Retroviruses push the envelope for mammalian placentation

Harmit Singh Malik[1]

Basic Sciences Division and Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA 98109

Retroviruses have had a tremendous, recurring impact on animal genomes. At least eight percent of the human genome is comprised of retroviruses at various stages of "fossilization" (1). These elements represent retroviruses that have directly infected genomes of germline tissues such that their imprints can now be passed on with the rest of the genome. Most insertions into host genomes are likely to (*i*) be instantly so deleterious that they are never passed on, or alternatively (*ii*) have very little consequence to host biology and be expected to abrade away via the accumulation of mutations (2). Although the large fraction of retroviral imprints show expected signatures of mutational degeneration, some retroviral genes have been surprisingly preserved against mutational inactivation. These represent instances in which host genomes have usurped some retroviral genes for their own use. Particularly intriguing are host domestications of retroviral envelope (*env*) genes. The best-known classes of these genes are the syncytin genes, which have been coopted by the host to mediate nutrient transfer from the mother to the developing embryo in eutherian mammals. In PNAS, Dupressoir and coworkers describe the oldest known domestication of retroviral envelopes represented by the *Syncytin-Car1* gene (3), which was domesticated at least 60 Mya, before the radiation of Carnivora. These results extend the range and age of syncytin domestications in mammals, but also raise intriguing questions about the biology underlying their recurrent invention.

Domestication of the syncytin genes represents a dramatic example of convergent evolution via the cooption of a retroviral gene for a key biological function in reproductive biology. In fact, syncytin domestication from a retroviral envelope gene has been previously shown to have independently occurred at least seven times during mammalian evolution (4–9). In the context of retroviral genomes, the *env* gene encodes a protein precursor that is cleaved into surface (SU) and transmembrane (TM) proteins that allow virions to attach to target cells and penetrate the cell membrane by fusion. SU protein serves to bind the virion to the host cell by interactions with receptors on the cell surface, whereas the TM protein serves to anchor the entire viral glycoprotein complex on the virion surface and to mediate the fusion of the virion with the host-cell membrane during entry. Many envelope proteins also encode a short peptide motif within the TM domain that has potent immunosuppressive activities, suppressing the production of cytokines and cell-mediated immunity (10). Intriguingly, these two activities—the abilities to mediate cell–cell fusion and evasion of the immune system—are key to the survival of the developing fetus in many mammals. The cell–cell fusion properties are essential for embryonic viability in mice (11) and prevention of pregnancy loss in sheep (12) because they allow the development of a placental syncytiotrophoblast layer that mediates transfer of nutrients and gases from the maternal blood. Retroviral envelope-associated immunosuppressive properties might help protect the fetal tissues from immune attack by the maternal immune system, although a requirement for this function has not yet been demonstrated.

Dupressoir and coworkers describe the oldest known domestication of retroviral envelopes represented by the *Syncytin-Car1* gene.

Three types of placental forms are found in eutherian mammals. Simian primates, rodents, and lagomorphs, in which syncytin genes have been described previously, all share the hemochorial type of placenta, in which fetal chorion is directly exposed to the mother's blood (13). A second, endotheliochorial form of placenta is also found among mammals including carnivores, wherein the fetal chorion is in contact with the endothelial wall of the mother's blood vessels (13). Despite the precedent of the seven separate syncytin genes (4–9), it was unclear whether mammals with endotheliochorial placentation also bear a syncytin. To address this question, the authors queried the completed genome sequences of cat, dog, and giant panda to identify any *env*-derived domesticated genes that had been preserved as intact ORFs (3). One gene appeared to have retained intact features of retroviral envelopes and was preserved in orthologous locations among the cat, dog, and giant panda genomes. Furthermore, this gene (*Syncytin-Car1*) had exclusive expression in the placenta, and in situ hybridization revealed that the expression pattern exactly coincided with the location where placental cells invade surrounding maternal tissues. Finally, pseudotyping experiments revealed that this particular envelope had retained fusogenic properties, i.e., it was able to confer infectious ability to an *env*-lacking recombinant retrovirus. Thus, a single envelope gene has been preserved in the same proviral context in the same genetic location since the common ancestor of dogs, cats, and bears. Detailed sequencing and analysis of *Syncytin-Car1* in 26 species representing all the suborders of Carnivora also revealed that the gene has been preserved largely under purifying selection since its birth (3). All evidence (e.g., maternofetal boundary placental expression, preservation of fusogenic activity) suggests that the *Syncytin-Car1* gene likely plays the same key role in placentation among all Carnivores first proposed in primate genomes and later demonstrated via genetic KOs in rodent genomes (3).

Ancient, Recurrent Evolution of Syncytins in Mammals

It is clear that the biological requirements for the hemochorial and endotheliochorial placentation are quite different yet both appear to use syncytins. Some eutherian mammals, like pigs and horses, also use a third type of placentation called epitheliochorial placentation, whereby the fetal chorion is in direct contact with the uterine epithelium (13). This third form lacks a syncytiotrophoblast and may not involve a syncytin-like gene at all. Phylogenetic reconstruction studies argue strongly that the most invasive (i.e., hemochorial) form of placentation is ancestral to all eutherian mammals (13). The finding of *Syncytin-Car1* also implies that at least two of four deep

Author contributions: H.S.M. wrote the paper.

The author declares no conflict of interest.

See companion article on pages E432 and 2206.

[1]E-mail: hsmalik@fhcrc.org.

branching eutherian mammal lineages encode syncytin-like genes (3). This suggests that the syncytiotrophoblast-containing form of placentation (and perhaps syncytin-like genes) evolved early in eutherian mammals.

How does one reconcile an early evolution of syncytin-mediated placentation in eutherian mammals with the remarkably recurrent cooption of different syncytin genes, from different retroviral lineages, at different points in the evolution of mammalian orders? For instance, *Syncytin-Car1* is at least 60 million years old and highly preserved, whereas the *syncytin-1* gene in primates is approximately 25 million years old, and preserved only in hominoids but not Old World monkeys (14). Furthermore, several syncytin genes are sometimes conserved in parallel in some mammalian genomes. For instance, rodents have both *syncytin-A* and *syncytin-B*, whereas some primates have at least *syncytin-1* and *syncytin-2* (some primates have even more envelope genes with placental expression). At least part of the explanation is that there is some partitioning of function among the independent syncytin domestications. In humans and mice, only one of the copies (*syncytin-2* in humans and *syncytin-B* in mice) has the immunosuppressive function (15). A second explanation is that the different syncytins are expressed in different layers of the placenta. In mice, *syncytin-A* and *syncytin-B* are expressed in the two separate layers of the murine syncytiotrophoblast (16). These findings raise the remarkable possibility that the various morphological innovations seen in placentation among different mammals (even among the hemochorial forms) may have been driven in part by the acquisition of different syncytin genes, with different receptors and different fusogenic abilities.

Why the Placenta?

The placenta is a unique organ that mediates a complex set of mother–offspring interactions over parental investment (17–19). Whereas an optimal evolutionary strategy for a mother's gene would be to allocate nutrients to each offspring to maximize her net reproductive success, each offspring (or more specifically the paternal genes in each offspring) would seek greater investment from the mother. Under this genetic conflict over maternal resources, an explanation for the recurrent cooption of syncytins over the course of evolution is that each new syncytin gene might provide yet another opportunity for a fetus to demand more in terms of maternal nourishment. The occasional signatures of positive selection that are seen in some syncytin genes, including in *Syncytin-Car1* (3, 14), may further reflect this mother–offspring conflict. The extent of genetic dissonance of interests between maternal and paternal genomes is affected by many ecological and life-history traits. For instance, multiple paternity and limited paternal investment in offspring accentuates the degree of conflict. It is possible that life-history transitions between "high" and "low" levels of conflict have driven the various transitions in placentation observed today in eutherian mammals, concomitant with a gain (or loss) of syncytin genes. Thus, the same retroviral genes that participate in host–viral interactions to determine infectivity, may also mediate maternal–offspring genetic conflicts in mammalian genomes.

The syncytin genes like the one identified in PNAS (3) thus serve as an important reminder of the evolutionary opportunism of host genomes while also providing a biological basis to explain some of the remarkable diversity of placentation in mammals. Viewing the recurrent invention of syncytin genes in the context of genetic conflict also serves to highlight the larger roles of genetic conflicts in shaping mammalian genomes and biology.

ACKNOWLEDGMENTS. A National Science Foundation CAREER Grant and an Early Career Scientist Award from Howard Hughes Medical Institute support my work on host-virus evolution.

1. Lander ES, et al.; International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. *Nature* 409:860–921.
2. Patel MR, Emerman M, Malik HS (2011) Paleovirology - Ghosts and gifts of viruses past. *Curr Opin Virol* 1: 304–309.
3. Cornelis G, et al. (2012) Ancestral capture of *syncytin-Car1*, a fusogenic endogenous retroviral *envelope* gene involved in placentation and conserved in Carnivora. *Proc Natl Acad Sci USA* 109:E432–E441.
4. Blond JL, et al. (2000) An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. *J Virol* 74: 3321–3329.
5. Mi S, et al. (2000) Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403:785–789.
6. Renard M, et al. (2005) Crystal structure of a pivotal domain of human syncytin-2, a 40 million years old endogenous retrovirus fusogenic envelope gene captured by primates. *J Mol Biol* 352:1029–1034.
7. Dupressoir A, et al. (2005) Syncytin-A and syncytin-B, two fusogenic placenta-specific murine envelope genes of retroviral origin conserved in Muridae. *Proc Natl Acad Sci USA* 102:725–730.
8. Heidmann O, Vernochet C, Dupressoir A, Heidmann T (2009) Identification of an endogenous retroviral envelope gene with fusogenic activity and placenta-specific expression in the rabbit: A new "syncytin" in a third order of mammals. *Retrovirology* 6:107.
9. Vernochet C, et al. (2011) A syncytin-like endogenous retrovirus envelope gene of the guinea pig specifically expressed in the placenta junctional zone and conserved in Caviomorpha. *Placenta* 32:885–892.
10. Haraguchi S, Good RA, Cianciolo GJ, Engelman RW, Day NK (1997) Immunosuppressive retroviral peptides: immunopathological implications for immunosuppressive influences of retroviral infections. *J Leukoc Biol* 61: 654–666.
11. Dupressoir A, et al. (2009) Syncytin-A knockout mice demonstrate the critical role in placentation of a fusogenic, endogenous retrovirus-derived, envelope gene. *Proc Natl Acad Sci USA* 106:12127–12132.
12. Dunlap KA, et al. (2006) Endogenous retroviruses regulate periimplantation placental growth and differentiation. *Proc Natl Acad Sci USA* 103:14390–14395.
13. Elliot MG, Crespi BJ (2009) Phylogenetic evidence for early hemochorial placentation in eutheria. *Placenta* 30:949–967.
14. Cáceres M, Thomas JW; NISC Comparative Sequencing Program (2006) The gene of retroviral origin Syncytin 1 is specific to hominoids and is inactive in Old World monkeys. *J Hered* 97:100–106.
15. Mangeney M, et al. (2007) Placental syncytins: Genetic disjunction between the fusogenic and immunosuppressive activity of retroviral envelope proteins. *Proc Natl Acad Sci USA* 104:20534–20539.
16. Dupressoir A, et al. (2011) A pair of co-opted retroviral envelope syncytin genes is required for formation of the two-layered murine placental syncytiotrophoblast. *Proc Natl Acad Sci USA* 108:E1164–E1173.
17. Trivers RL (1974) Parent-offspring conflict. *Am Zool* 14: 249–264.
18. Haig D (1993) Genetic conflicts in human pregnancy. *Q Rev Biol* 68:495–532.
19. Crespi B, Semeniuk C (2004) Parent-offspring conflict in the evolution of vertebrate reproductive mode. *Am Nat* 163:635–653.