—Mini Review—

The Evolution of the Placenta and Viviparity is Related to LTR Retrotransposon-derived Genes in Mammals

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Abstract: The two LTR retrotransposon-derived genes *PEG10* and *PEG11*/*RTL1* play essential roles in placental formation and its maintenance in the current mammalian developmental system. The former is a therian-specific and the latter is a eutherian-specific gene, suggesting that once they had been acquired in common ancestors of the therian and eutherian mammals, respectively, they have underwent positive selection during the course of mammalian evolution due to developmental advantages they conferred. Thus, their exaptation had a profound impact on the evolution of mammalian viviparity as well as the emergence of the therians and eutherians, the infraclass and subclass of mammals, respectively. How the exaptation of *PEG10* and *PEG11*/*RTL1* come to take place in the mammalian lineage? We propose that the exaptaion mechanism comprises two subsequent steps. The first follows the pattern of nearly neutral theory of molecular evolution, where the retrotransposons were neutralized by DNA methylation and genetic drift fixed them in the population. At the next step, Darwinian evolution propagated these genes into all the therian and eutherian population by natural selection. In the course of these processes, the placenta, a mammalian-specific extraembyonic tissue, may have been of special importance as a sort of "natural laboratory" for mammalian evolution.

Key words: LTR retrotransposon-derived genes, Mammalian evolution, Exaptation, Placenta, Viviparity

Introduction

Brosius and Gould coined the term "exaptation" to describe the evolutionary mechanism by which novel genes are produced from other genetic materials that have different roles in other organisms [1]. A recent comprehensive survey of the mammalian genomes revealed the presence of 12 newly acquired genes that are derived from a sushi-ichi-related LTR retrotransposon in eutherians and marsupials, the *SIRH*(sushi-ichi-related retrotransposon homologues) [2, 3], *MART*(mammalian-specific retrotransposon transcripts) [4] or *SUSHI* families of genes [5]. We previously demonstrated that the *Peg10*(*Sirh1*) and *Peg11/Rtl1*(*Sirh2*) genes play essential roles in the formation and maintenance of the placenta in mice [2, 6]. *PEG10* and *PEG11* are therian- and eutherian-specific genes, respectively, indicating they have been positively selected due to their advantageous functions in mammalian development once they were exapted as newly acquired genes in therian and eutherian ancestors, respectively. Thus, *PEG10* and *PEG11/RTL1* are very good examples of the exaptation as well as macroevolution that takes place in Darwinian evolution [7, 8].

In this article, we will discuss what *Peg10* and *Peg11/Rtl1* tell us about mammalian evolution and how they were most probably exapted. We would like to present one likely scenario of how their exaptation occurred in the course of mammalian evolution and consider the role of nearly neutral theory of evolution in the functional adaptation of mammals in the Darwinian theory of evolution. We emphasize the placenta as a site in which exaptation occurred [7, 8].

Two LTR Retrotransposon-derived Genes, *PEG10* and *PEG11/RTL1,* Play an Essential Role in Mammalian Development

It is well known that parthenogenetic mouse embryos having two maternally-derived genomes die around day 9.5 because of poor placental development [9–11]. This suggests paternally expressed gene (s) plays an impor-

Received: October 3, 2012
Accepted: November 14, 2012
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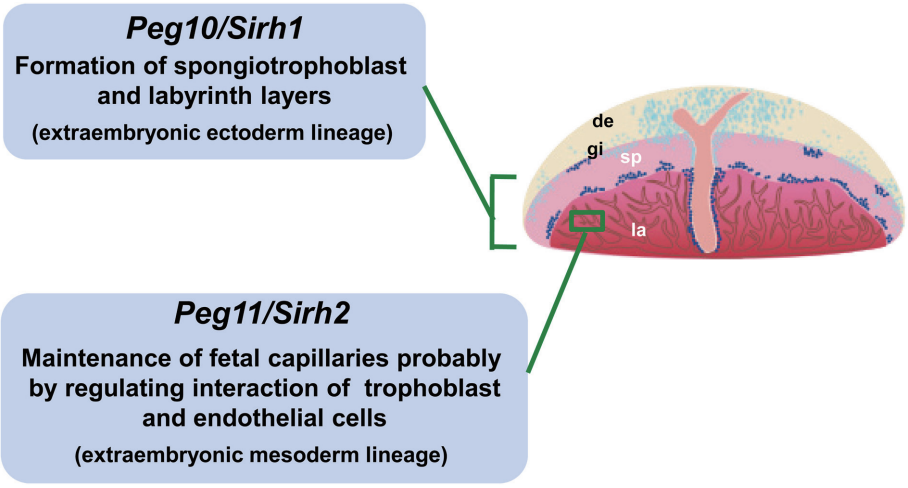

Fig. 1. Essential placental function of *Peg10* and *Peg11/Rtl1*.
Peg10 is responsible for formation of spongiotrophoblast and labyrinth layers that are essential for placental function in mice. In the latter, fetal capillaries are bathed in maternal blood, and exchange of gases and nutrients occurs between fetal and maternal blood. *Peg11/Rtl1* is essential for maintenance of the fetal capillaries, the feto-maternal interface. la; a labyrinth layer, sp: a spongiotrophoblast layer, gi: a giant trophoblast layer, de: maternal decidua.

tant role in mammalian development and help determine the fate of the embryos. Mouse proximal chromosome 6 is the only reported imprinted region in which maternal duplication results in early embryonic lethality [12]. In 2001, we identified human *PEG10* on human chromosome 7q21, an orthologous region of mouse proximal chromosome 6, as a candidate gene for this parthenogenetic death [13]. We finally demonstrated that *Peg10* KO mice exhibit early embryonic lethality with severe placental defects similar to the parthenogenetic embryos, and concluded that *Peg10* is one of the major imprinted genes responsible for the early embryonic lethality caused by the maternal duplication of proximal chromosome 6 [2]. *Peg10* KO embryos exhibited development to at most 9.5 days of postcoitum (dpc) and their placenta lacked two essential functional parts that support embryonic growth and development, the labyrinth and spongiotrophoblast layers, almost completely (Fig. 1).

Mouse distal chromosome 12 and its orthologous human distal chromosome 14 also harbor critically important imprinted regions in both mouse and human development [12, 14, 15]. The maternal duplication of chromosome 12 in mice causes late embryonic/neonatal lethality associated with growth retardation, and its paternal duplication causes late embryonic lethality associated with growth and morphological abnormalities. In humans, similar abnormal phenotypes are observed in patients with maternal and paternal disomies of human chromosome 14 (upd (14) mat and upd (14) pat), respectively [16, 17].

Ovine *PEG11* was reported in the course of a study on callipyge mutations responsible for the onset of muscle hypertrophy related to this imprinted region [18]. In 2008, we demonstrated that *Peg11/Rtl1* KO mice display late embryonic/neonatal lethality associated with growth retardation due to placental malfunction [6]. In the placenta, clogging was observed in fetal capillaries in the labyrinth layer and in addition, phagocytosis of the fetal capillary endothelial cells by surrounding trophoblast cells was observed in these regions (Fig. 1). The resulting reduction of blood flow to the embryos seems to be the direct cause of the evident late embryonic lethality and growth retardation. We also reported that overexpression of *Peg11/Rtl1* causes a different type of alteration of fetal capillaries that leads to neonatal lethality, like the case of the mice with the paternal duplication of distal chromosome 12 [6]. Thus, it is concluded that *Peg11/Rtl1* is a one of the major imprinted genes responsible for the phenotypes caused by maternal and paternal duplications of distal chromosome 12. Similarly, lack and overproduction of *PEG11/RTL1* are attributable to the human upd (14) mat and upd (14) pat phenotypes, respectively [19].

Interestingly, *PEG10* and *PEG11/RTL1* have a high degree of homology with a sushi-ichi retrotransposon.

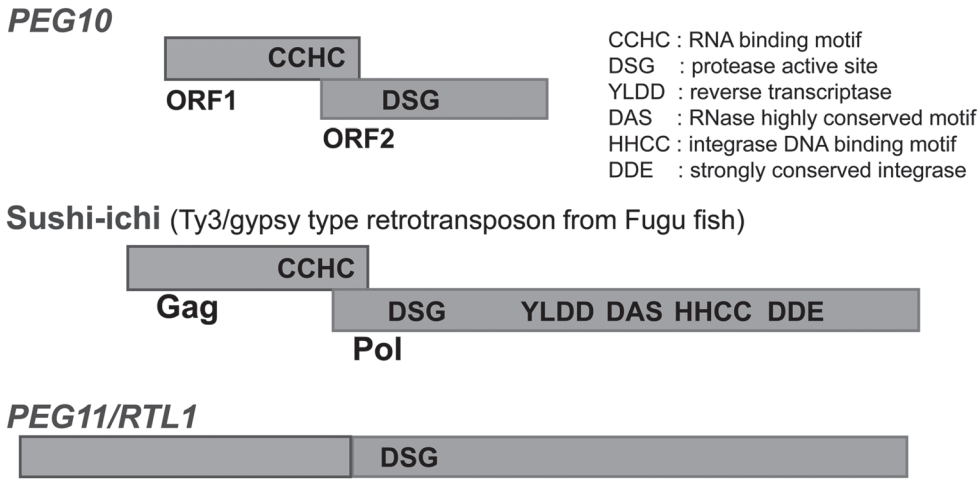

Fig. 2. PEG10 and PEG11/RTL1 proteins display significant homology to Gag and Pol proteins of a suchi-ichi retrotransposon.
Several features of LTR retrotransposons are retained in PEG10 and PEG11/RTL1 proteins, such as a CCHC RNA binding motif in Gag, a DSG protease active site in Pol and a -1 frameshift mechanism to produce a Gag-Pol fusion protein in the former and the DSG protease active site in the latter. Overall amino acid sequence of both PEG10 and PEG11/RTL1 proteins display 20–30% homology to Gag and Pol proteins.

PEG10 has two open reading frames (ORF1 and ORF2), which display 20–30% homology to the Gag and Pol proteins of the sushi-ichi retrotransposon, respectively [13] (Fig. 2). The PEG10 ORF1 has a CCHC RNA binding motif in the Gag protein and the PEG10 ORF2 has a DSG protease active site in the Pol protein. Importantly, a -1 frameshift mechanism that produces a Gap-Pol fusion protein unique to the LTR retrotransposons and retroviruses is also conserved in *PEG10,* and a PEG10 ORF1-2 fusion protein has been demonstrated in the placenta [2, 20, 21]. The ovine PEG11 protein was also reported to show an overall homology with the sushi-ichi retrotransposon Gag and Pol proteins [18, 22] (Fig. 2), and thus *PEG11* subsequently came to be called *RTL1*(retrotransposon-like 1). The DSG protease active site is also conserved in the PEG11 protein. All of these features together provide strong evidence that *PEG10* and *PEG11/RTL1* are derived from a sushi-ichi-related LTR retrotransposon.

The Contributions of *PEG10* and *PEG11/RTL1* to the Evolution of Mammalian Viviparity as Therian- and Eutherian-specific Genes, Respectively

A comparative genome analysis of eutherians, marsupials, monotremes and non-mammalian vertebrates revealed that *PEG10* is only conserved in the therian mammals. Its presence has been confirmed in more than 20 eutherian species and 3 marsupial species, including both Australian and South American marsupials, while no *PEG10* orthologue was found in the platypus, an Australian monotreme species as well as birds, reptiles, amphibians and fish [23] (unpublished data). Therefore, it is evident that the insertion of the original *PEG10* retrotransposon occurred in a common therian ancestor and its exaptation was completed before the spilt of the marsupials and eutherians [7, 23] (Fig. 3). Both the eutherians and marsupials are viviparous mammals using a placenta in support of embryonic development and growth during the gestation period. Thus, it may be said, as far as *PEG10's* function is concerned, the exaptation of *PEG10* is a critical milestone in the history of mammalian viviparity.

In contrast, *PEG11/RTL1* is a eutherian-specific gene, that is, it is conserved in only the eutherians and not the marsupials (Fig. 3). Interestingly, the eutherians and marsupials have different types of placenta, a chorioallantoic placenta and choriovitellin placenta (i.e. a yolk sac placenta), respectively, and adopt different reproductive strategies. The marsupials give birth to relatively tiny and altricial young after a short gestation and the pups keep developing in the mother's pouch for long time by means of lactation, while the eutherians give birth to more ma-

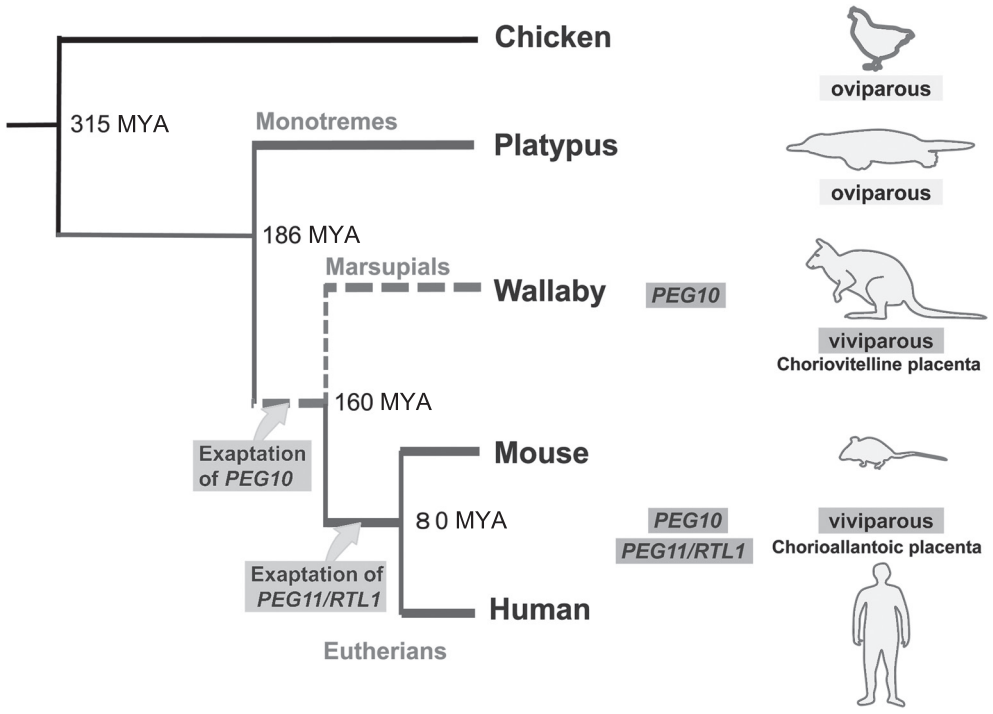

Fig. 3. Exaptation of *PEG10* and *PEG11/RTL1* in mammalian evolution.
PEG10 is conserved only in the therian mammals and *PEG11/RTL1* is a eutherian-specific gene. Thus, their exaptation must have occurred in ancestors of the therian and eutherian mammals, respectively. MYA: million years ago.

ture, precocial young after a long gestation [24]. It is highly likely that the placental type was one important factors in the reproductive strategy followed. It should be noted that *PEG11/RTL1's* function is essential for the long gestation that takes place in the eutherians, suggesting that *PEG11/RTL* is also a key contributor to the establishment of the eutherian reproductive system [6].

A Possible Scenario for Retrotransposon Exaptation in Mammals

As described, *PEG10* and *PEG11/RTL1* are instances of exaptation from an LTR retrotransposon as well as positively selected genes in Darwinian evolution after their exaptation [7]. It is in accord with evolutionary theory to think that they propagated in the therian and eutherian population under natural selection due to the developmental advantages they conferred. A question arises: what happened to the inserted original retrotransposons in the ancestors' genomes before exaptation? Given that inserted retrotransposons and retroviruses are totally inactivated by heavy DNA methylation on their promoter regions in the host mammalian species because they are harmful, it is highly likely that the inserted retrotransposons in the common therian ancestor would also have been repressed by such DNA methylation. In such conditions they would have been neutral genes. According to the neutral theory of molecular evolution proposed by Kimura [25], such neutral genes be transmitted to the next generations and became fixed in a given population by chance, a process known as "genetic drift". In the case of *PEG10*, the exaptaion must have occurred within a period of approximately 26 million years, from the diversification of the therians from the monotremes which took place 186 million years ago (MYA) to the split of the two therian groups in 160 MYA (Fig. 3). Because, the amino acid homology of *PEG10* and the sushi-ichi retrotransposon is only 20–30%, the inserted original sushi-ichi related retrotransposon must have been subjected to a number of mutations in this period that finally resulted in the *PEG10* prototype that conferred some slight advantage. Then, Darwin evolution took over and *PEG10* became more advantageous by positive selection and, after that, came to be conserved in all of the therian species as a result of purifying selection [4, 22] (Fig. 4).

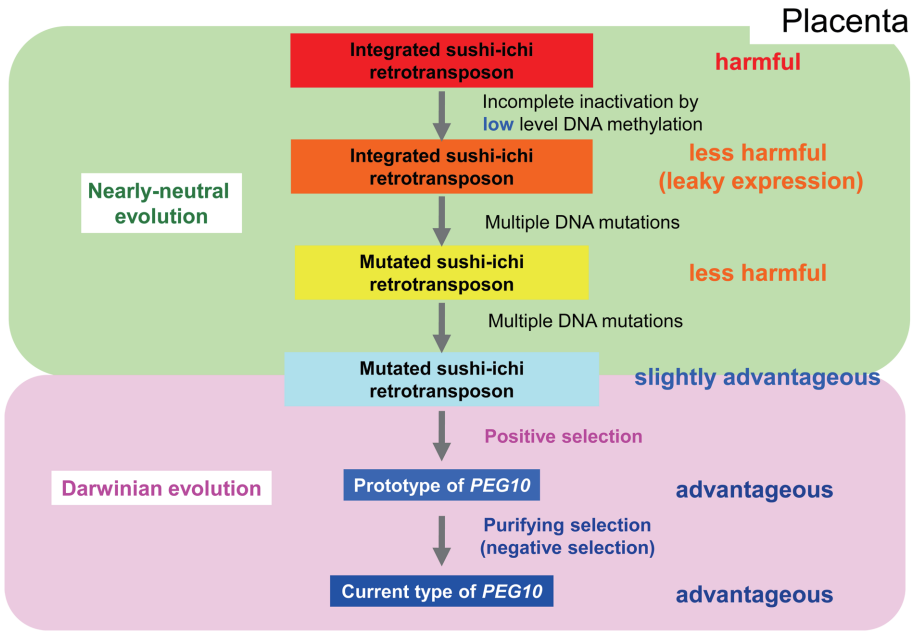

Fig. 4. Two subsequent steps in exaptation from LTR retrotransposons in placenta.
In this scenario, both nearly neutral evolution and Darwinian evolution contribute to the exaptation mechanism from LTR retrotransposons. We assume that placenta has been providing an unusually suitable place for exaptation due to its lower DNA methylation level, and served as a sort of natural laboratory for mammalian evolution in which a numbers of new genes were acquired for certain mammalian-specific traits.

The Placenta as a Laboratory for Mammalian Evolution

Ohta extended Kimura's neutral theory of molecular evolution to her own "nearly neutral" theory, and predicted that less harmful as well as strictly "neutral" mutations could become fixed in a population provided the population size were small enough [26]. It is known that the DNA methylation levels in extraembryonic tissues, such as the yolk sac and placenta, are lower than those in other embryonic and adult tissues, so the retrotransposons and retroviruses in these tissues are not completely repressed. In this situation, the integrated retrotransposons would be less harmful in terms of leaky expression (Fig. 4). Importantly, placental cells would have retained the function of retrotransposon-derived genes from the time their integration and benefit when the function conferred some slight advantage. At this point, a swift transition from the state of nearly neutral evolution to that of Darwinian evolution would take place. Thus, we assume that the placenta was an unusually suitable site for exaptation, or, in other words, it served as a sort of natural laboratory for mammalian evolution in which a number of new genes were acquired that were of special importance for certain mammalian-specific traits [7, 8].

Discussion

Dozens of newly acquired genes have been shown to be derived from LTR retrotranspsons and retroviruses in mammals. As described in this article, the *SIRH* family of genes comprises 12 genes, and another LTR retrotransposon-derived gene family, the *PNMA*(paraneoplastic Ma antigen) family, comprises 19 and 15 genes in humans and mice, respectively [27, 28 and unpublished data]. The *SASPase* gene is a single mammalian-specific gene encoding skin aspartic protease (SASPase), which is known to be a retroviral-like aspartic protease that plays a key role in determining the texture of skin by modulating the degree of hydration via the processing of profilaggrin [29–31]. The SCAN-family is not a mammalian-specific gene family, because its ancestral form exists in non-mammalian vertebrates, but its expansion has been confirmed in the eutherians. Approximately 60 and 40 genes are known in humans and mice, respectively, and some of them are involved, as transcription factors,

in development and differentiation [28]. The *SYNCYTYIN* genes were exapted independently in many eutherian species and are derived from retroviral *Env* genes [32–34]. We propose that the placenta has been a site of retrotransposon exaptation during the course of mammalian evolution, which is consistent with the fact that *Peg10* and *Peg11/Rtl1,* as well as the retrovirus-derived *Syncytin A* and *B* genes [2, 6, 35, 36], play essential roles in the normal, healthy placenta [7]. However, this does not necessarily mean that independent exaptation events have happened as for each occurrence of these genes. Some might have been produced by the duplication of a single originally exapted gene, such as in the case of the SCAN family genes. Approximately 8% of the human genome is composed of some 450,000 copies of LTR retrotransposons and endogenous retroviruses (ERVs), although thus far none have been shown to have transposable activity. Therefore, we can say that retrotransposon exaptation is evidently quite a rare event, but once it takes place, its impact is enormous. *PEG10/SIRH1* is common to both the marsupials and eutherians, while *PEG11/SIRH2* and *SIRH3-11* are only found in the eutherians, and *SIRH12* was derived from a marsupial-specific retrotransposition event [13]. Therefore, the eutherians and marsupials have completely different sets of *SIHR* genes, except for *PEG10*. The same is also true for the *PNMA* genes: most are eutherian-specific and a few are marsupial-specific (in preparation). Therefore, the functions of other *SIRH* genes and *PNMA* genes are of special interest to elucidate mammalian evolution. It is possible that each *SIRH* and *PNMA* gene plays some mammalian-specific functional role in gestation, delivery and maternal nursing behavior, including lactation, thus contributing to the establishment and the diversification of the marsupial and eutherian reproductive systems.

It is highly likely that DNA methylation played an essential role in the exaptation of retrotransposons in the evolution of the therian mammals. We assume that the evidence indicates that nearly neutral evolution played an essential background role in the exaptation mechanism by both inactivating and neutralizing integrated retrotransposons. Neutral evolution is widely accepted to play an important role in the molecular evolution that is related to changes at the DNA level. However, its contribution to evolutionary changes at morphological, functional and behavioral levels is still under debate. It seems logical to think that the exaptation mechanism comprises two subsequent steps: the first step is in accord with the processes of nearly neutral evolution, while the second step depends upon natural selection. Therefore, it appears there is good evidence that nearly neutral evolution makes a contribution to phenotypic (functional) changes via the exaptation mechanism as well as by the diversification of new genes occurring after gene duplication originally proposed by Kimura [37].

How did the mammalian viviparous reproductive system originally start using the retrotransposon-derived *PEG10* gene? If the viviparous reproductive system indeed first happened in a single individual, it is very difficult to imagine that such an individual would survive and propagate offspring. Kimura first advance the notion that the neutral theory of molecular evolution helped explain how new species originated from a population subset [37]. Because preadaptive mutations were already distributed, albeit in a neutral manner, adaptive functions could emerge in such a group at the same time when the selective pressures came to be changed by the inhabitation of a new environment. In this regards, it might be useful to say that the neutral evolution process acts as a “capacitor” for evolutionary changes at the morphological, functional and behavioral levels [38, 39].

Acknowledgements

We thank all the collaborators and laboratory members, especially Ryuichi Ono, Yoich Sekita and Shunsuke Suzuki for analyzing *Peg10* and *Peg11* KO mice and marsupial *PEG10*, respectively. The work has long been supported by a number of grants, Grants-in-Aid for Scientific Research (S) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan (FI), Funding Program for Next Generation World-Leading Researchers (NEXT Program)(TK-I) and Bilateral Program on Joint Research Project (FI), Creative Science Research (FI and TK-I) from the Japan Society for the Promotion of Science (JSPS), Asahi Glass Foundation (TK-I), The Mitsubishi Foundation and the Uehara Memorial Foundation (FI).

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