

Defining the function of a prolactin gene family member

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Mouse molecular geneticists have been frustrated to find that knockout alleles do not always produce a phenotype.

Prince and Pickett (1)

The quotation above is a considerable understatement for all of those graduate students and postdoctoral researchers whose hopes for scientific glory, or at least a diploma, were resting on a phenotype resulting from the ablation of their gene of interest. The source of this consternation is functional redundancy, and paralogous gene families are at least one source of such redundancy. The perpetuation and expansion of gene family members is likely caused by a selective advantage that such paralogous genes provide a species in dealing with the myriad of environmental and physiological challenges (nutrient availability, predatory pressure, disease, genetic conflict, etc.) that are confronted by animals in the wild. Animals maintained in the relatively aseptic environment of modern housing facilities are not exposed to such challenges. Under these conditions, the absence of a gene family member may not produce an overt phenotype. A report in this issue of PNAS by Ain *et al.* (2) describes just such an occurrence after disruption of the gene for a prolactin (PRL) family member, PRL-like protein A (PLP-A). Despite the initial lack of a phenotype, they were able to gain some insight into the biological role of PLP-A by providing an appropriate environmental challenge.

Gene Families

All animals possess numerous duplicated genes. In fact, the rate at which gene duplications occur is quite high and is comparable to the rate at which point mutations arise per nucleotide site (3–5). It is perhaps not surprising that gene duplication has long been suggested to provide the raw materials on which adaptive evolution can act (6). Indeed, expansion of gene families is often a species-specific phenomenon, with certain gene families restricted to closely related species or single phylogenetic orders (7–9). Speciation itself could even be a natural outcome of gene duplications (3, 10).

What, then, is the fate of duplicated genes? Most of the time, one member of a duplicated pair is functionally silenced in a relatively short time (3). However,

in some cases gene duplications are retained and the pairs can acquire either novel functions (neofunctionalization) or partitioned functions (subfunctionalization) compared to the ancestral allele (11). Subsequent gene duplications often follow, resulting in the establishment of paralogous gene families (12). Presumably, such genes are retained and propagated because they convey a selective benefit, although the specifics regarding how and why some duplicated genes become fixed in a population is the subject of much debate (13, 14). In any event, for a period after duplication, the gene pairs will possess overlapping functions,

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particularly if transcriptional regulatory sequences also are duplicated. This functional redundancy probably diminishes over time as the duplicated genes become more refined in their new roles.

What, then, is the role of duplicated genes? Perhaps insight can be gained by identifying the cell types and tissues known to express them. In mammals, recent expansions have occurred disproportionately in genes involved in immune function and reproductive organs such as testes, epididymis, and placenta (4, 12). Gene families expressed by the immune system make sense intuitively because they would provide the genetic resources to adapt to an ever-changing array of pathogens. In contrast, the presence of gene families expressed in reproductive tissues is not so clear. It has been suggested that genetic conflict can occur between the mother and fetus (e.g., their respective interests in nutrient partitioning often differ) as well as between males competing for mates (15, 16). In other words, a genetic “arms race” seems to be taking place between individuals within a species that may be somewhat reminiscent of, but more subtle than, that occurring in host–pathogen interactions.

Gene Families Expressed at the Placental–Uterine Interface

Investigations of gene families expressed predominantly, or exclusively, in the placenta have been ongoing for some time (7, 17, 18). The physiological roles of the placenta are rather conserved. The placenta acts to transmit nutrients and waste, modulate the maternal immune system (to protect the antigenically distinct fetus), and coordinate maternal and fetal physiology via an array of bioactive compounds and hormones (19, 20). Despite these common roles, placentas vary dramatically in gross shape and in the intimacy of contact with maternal tissues (21). For example, in rodents and great apes the discoid placenta invades aggressively into the uterine endometrium, breaching maternal blood vessels, until the trophoblasts (outer cell type of the placenta) are directly bathed in maternal blood (20). At the other extreme is the diffuse placenta of the pig, in which no erosion of the uterine lining occurs (21). Carnivores and ruminant ungulates possess placental types that are intermediate between these extremes (21). Such diversity leads to rather striking differences in the endocrinological, immunological, and metabolic interactions that take place between the fetus and mother. Consequently, the selective pressures operating on placental genes would also be predicted to be distinct between species.

One would predict that the diversity among placentas is a reflection of differences in gene expression, either from novel expression patterns of conserved genes, the acquisition of novel genes, or both. This assumption indeed seems to be the case because species-specific gene families have been identified that are unique to each placental type (17, 18, 22–24). Many members of the rodent PRL/growth hormone gene family also are expressed at the placental–uterine interface. These genes represent one of the more extreme examples of the complexity and diversity that can arise in gene families. They are also an example of how paralogous genes can be selected to fulfill a range of distinct, but often overlapping, functional roles.

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The Rodent PRL Gene Family

To date, there are 26 known mouse PRL family members (7). The PRL family members have been divided into those with biological activities that are considered classical (five members) and those that are nonclassical, based on whether they are functioning through the PRL receptor or through other signaling pathways, respectively. The major targets for those mouse PRL members acting through the classical pathway are the corpus luteum and mammary tissue, although the brain, pancreas, and immune system also are targeted (7). The receptor systems for the remaining members of the mouse PRL family either have not been characterized or have been shown to act through distinct receptor systems.

The PRL family members exhibit a range of temporal and spatial expression patterns among the different trophoblast lineages and the uterine decidua (differentiated stroma cells along with various types of immune cells) (7). The identification of cellular targets has, in some cases, permitted putative functions to be defined (summarized in ref. 7). From these preliminary studies it is clear that the diversity of the uteroplacental PRL family also is reflected in the range of cellular targets on which they act.

Ain *et al.* (2) sought to provide a better clarification of the role of one of the PRL gene family members, PLP-A, by inactivating the PLP-A gene. Upon doing so, the resulting phenotype was indistinguishable from that of WT animals. In light of the functional redundancy that is a natural outcome of gene duplication, such a result was perhaps not unexpected.

The target cells recognized by PLP-A are uterine natural killer (NK) cells residing in the mesometrial uterine decidua (the side from which the uterine blood supply enters) (25, 26). These cells have been shown to be intimately associated with the uteroplacental vasculature (27). Others also have suggested that the primate PRL/growth hormone members expressed in the placenta permit adaptations to metabolic stress (28). Therefore, Ain *et al.* (2) reasoned that the PLP-A protein, acting via uterine NK cells, probably was involved with remodeling or proper functioning of the uteroplacental vasculature. To test their hypothesis, they provided a metabolic stress appropriate to those observations: hypoxia. The mice were subjected to hypobaric hypoxic conditions, equivalent to breathing 11% oxygen, between days 7.5 and 12.5 of gestation (the time at which PLP-A expression and uterine NK cell density are maximal). WT animals were able to compensate for the decreased oxygen, whereas the knockout animals failed to do so. In the PLP-A knockout animals, the trophoblasts were unable to grow into the mesometrial decidua, which resulted in a failure to establish an adequate utero-placental vascular exchange. Severe growth retardation of the conceptuses was apparent by day 9.5 (2 days after hypoxic conditions were initiated); by day 11.5, nearly all of the conceptuses were resorbed.

The inability of the knockout animals to adapt to the hypoxia confirmed that at least part of the function of PLP-A is in facilitating modifications of the uteroplacental unit in response to metabolic challenges. The reason the PRL paralog, PLP-A, has been preserved in the rodent genome is almost certainly

not in helping rodents to survive in a severely low-oxygen environment. Rather, the hypobaric hypoxia challenge was administered as a rather rigorous test of the ability of the PLP-A knockout animals to respond to a stress placed on the uteroplacental vasculature. Additional work, perhaps with more subtle environmental challenges, will better define the role of PLP-A in rodent placentation.

Conclusion

The study of species-specific gene families may well provide insights into the genetic events involved in speciation, host-parasite interactions, and reproduction/development. However, assigning specific functions to the members of a gene family by genetic models can be an exceedingly difficult endeavor amid the background of functional redundancy. Investigators confronted with a lack of an apparent phenotype (whether it is caused by redundancy in gene families or the "cross-talk" inherent in signal transduction systems) may need to consider the use of an appropriate challenge to illuminate the importance of a particular gene. Unfortunately, deciding which environmental or biochemical challenge to perform may not be entirely intuitive. It falls to the investigators to use their knowledge of the target protein's expression pattern, intracellular location, associated proteins, and target cells (in the case of extracellular ligands) to come up with creative ways to provide an environmental challenge that will shed light on the biological role of their target gene.

Based on the results of Ain *et al.* (2), perhaps all of those knockout rodent lines out there that are lacking a phenotype deserve another look.

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