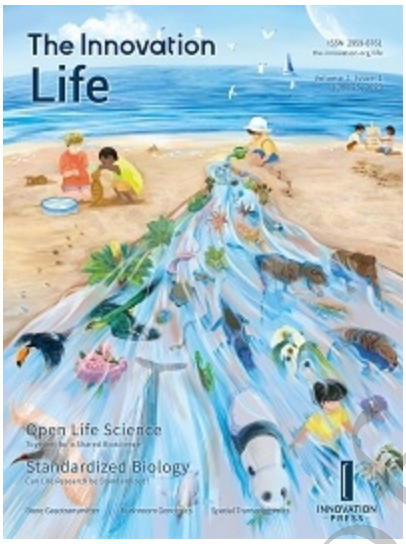

Beyond junk DNA: Adaptive evolution of pseudogenes in vertebrates

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Beyond junk DNA: Adaptive evolution of pseudogenes in vertebrates

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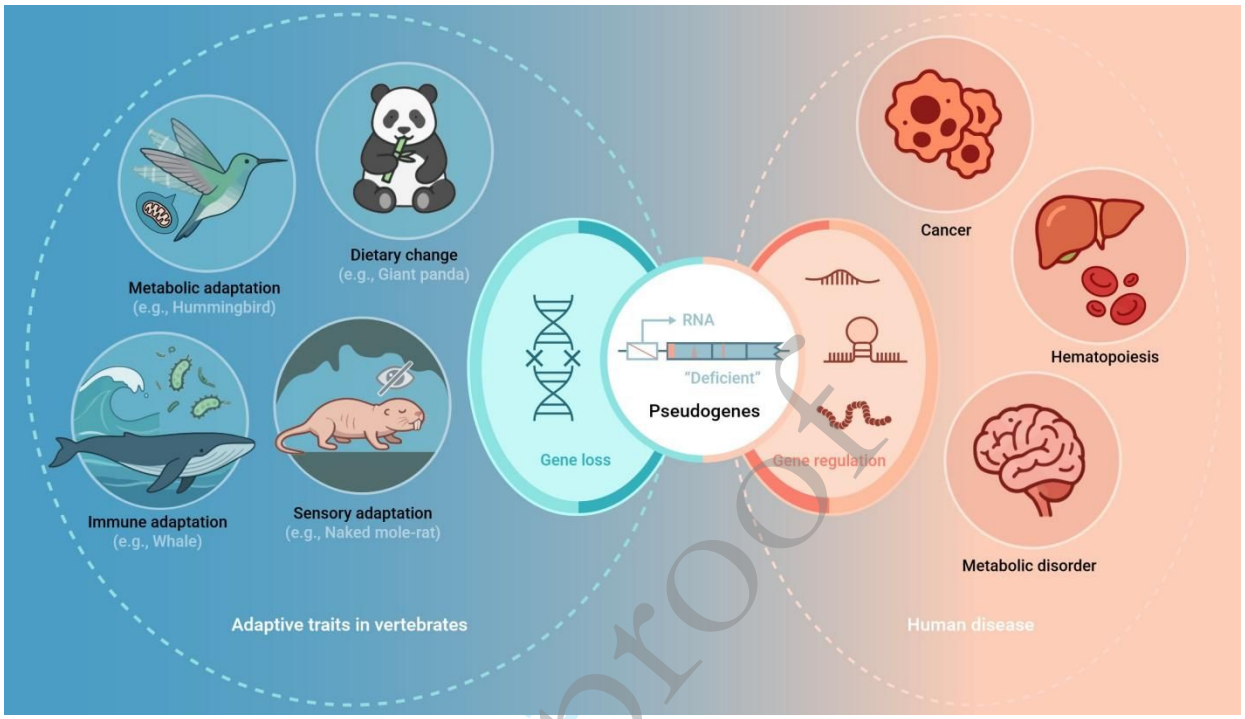
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Pseudogenes are now recognized as dynamic contributors to vertebrate adaptation and human disease.
- Recurrent pseudogenization events shape immune, sensory, and metabolic adaptation in vertebrates.
- In humans, pseudogenes play protective or pathogenic roles in cancer, hematopoiesis, and metabolic disorders.

ABSTRACT

Pseudogenes, once regarded as genomic fossils or “junk DNA”, have emerged as important players in adaptive evolution of animals and regulation of human disease. In this review, we synthesize recent advances in understanding the evolutionary dynamics and functional implications of pseudogenes across vertebrates. We firstly focused on the adaptive evolution of pseudogenes in vertebrates, highlighting how pseudogenization-derived gene loss contributes to lineage-specific adaptations in diverse habitats, including immune, sensory, dietary changes, sex determination and metabolic traits. Convergent pseudogenization events have been documented across multiple species facing similar ecological challenges. We then summarized the functional roles of pseudogenes in human diseases, highlighting their dual capacity to act as either protective regulators or pathogenic drivers in cancer, hematopoiesis and metabolic disorders. These pseudogenes frequently act through non-coding networks, translation of truncated peptides, or cis-regulatory DNA elements. These advances demonstrate that pseudogenes are not passive relics but active regulators of gene expression networks. Finally, we propose that integrating long-read RNA-seq data and using CRISPR/Cas9 to test causal variants represent key future directions for dissecting pseudogene functions in evolution and disease.

Keywords: pseudogenes, adaptive evolution, vertebrates, human disease, convergent evolution

INTRODUCTION

Pseudogenes are typically defective gene copies disabled by mutations, such as premature stop codons, frameshifts, or deletions. Once regarded as mere evolutionary remnants or “junk DNA”, these gene copies have gained significant scientific interest due to their potential functional roles and evolutionary insights. These non-functional sequences arise from gene duplication, retrotransposition, or gene decay and exhibit intriguing evolutionary dynamics. The study of pseudogenes provides valuable perspectives on genome evolution, gene regulation, and the functional diversity of genomes across various species.

Pseudogenes are classified into four major categories: processed, unprocessed, unitary, and polymorphic (Figure 1). Processed pseudogenes result from retrotransposition events where mRNA is reverse-transcribed and inserted back into the genome, lacking introns and regulatory elements.[1] Unprocessed pseudogenes originate from gene duplication followed by mutations that lead to loss of function, retaining intronic and regulatory structures.[2] Unitary pseudogenes, however, are formed when a functional gene directly acquires disabling mutations without duplication.[3] This process reflects gene loss through pseudogenization, where an initial loss-of-function mutation triggers gradual mutational decay rather than abrupt physical removal of the gene.[4] The last type is polymorphic pseudogenes, which appear disrupted in the reference genome but may remain intact in some individuals. The evolutionary significance of pseudogenes lies in their contribution to genomic innovation and complexity. They act as raw materials for the evolution of new genes and regulatory elements.[5] Pseudogenes can influence the expression of their functional counterparts through various mechanisms, including acting as decoys for regulatory molecules or sources of novel regulatory RNA.[6] Moreover, their presence can reflect evolutionary pressures and genomic stability within a lineage.[7]

Recent advances in genomic technologies have facilitated the identification and functional annotation of pseudogenes across diverse taxa. Comparative genomic studies reveal that pseudogene content varies significantly among species, reflecting different evolutionary histories and selective pressures.[8] For instance, the human genome harbors approximately 14,000 pseudogenes, highlighting their prevalence and potential roles in human biology.[9] Furthermore, the study of pseudogenes has uncovered their involvement in disease processes. Pseudogene expression profiles are altered in various cancers, suggesting their potential as biomarkers and therapeutic targets.[10] Understanding the evolutionary pathways and functional impacts of pseudogenes is crucial for elucidating their roles in health and disease. Pseudogenes are not merely genomic fossils; they are also active participants in genome evolution and function. These studies offer profound insights into the mechanisms driving genetic diversity, regulatory innovation, and evolutionary adaptation. In this review, we summarize the advances of pseudogenes' evolution in vertebrates, proposing perspectives for understanding their roles in adaptive evolution of species and human disease.

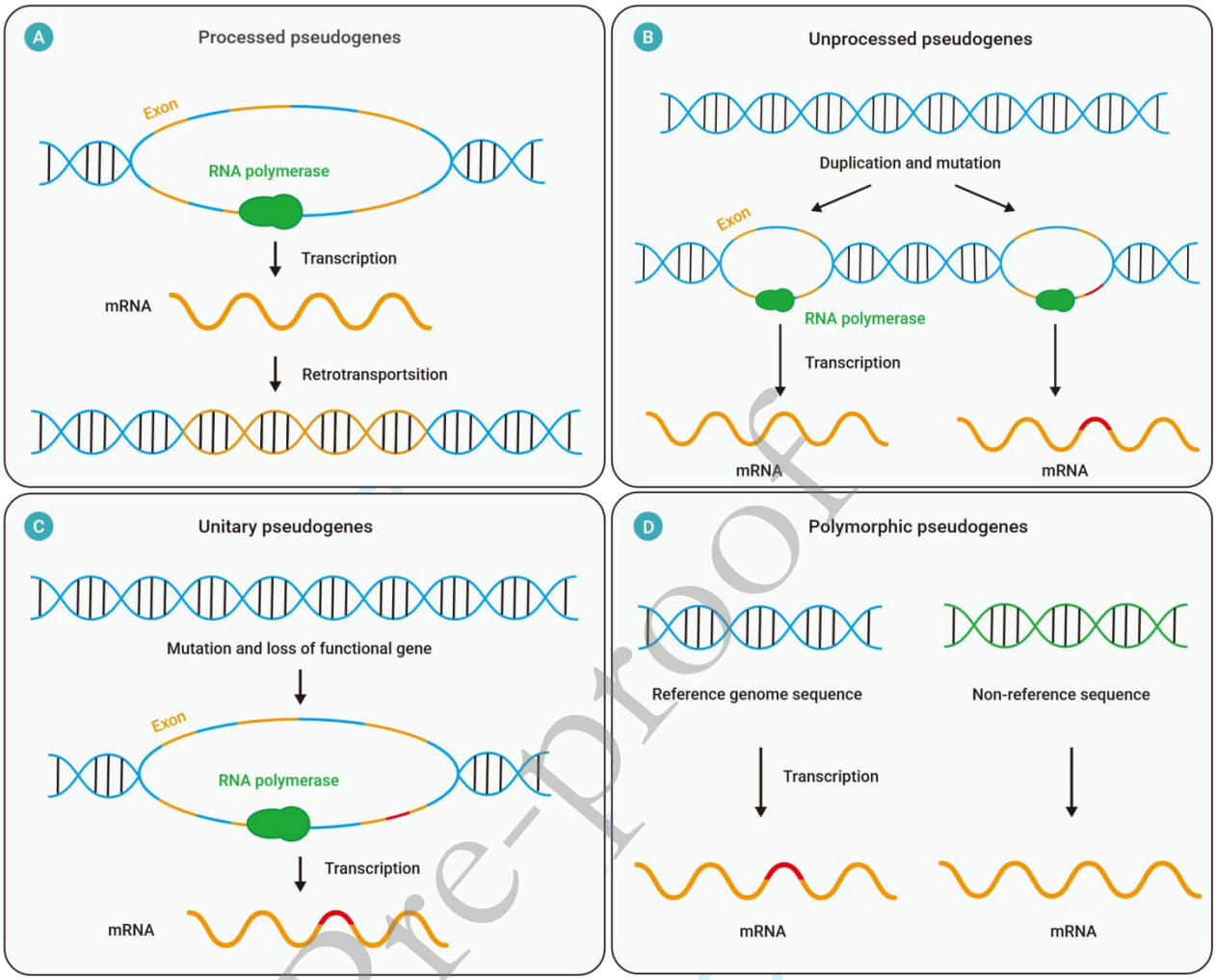


Figure 1. Major classes of eukaryotic pseudogenes (A) Processed pseudogenes are derived from reverse transcription and integration of mRNA; (B) Unprocessed pseudogenes arise from gene duplications that accumulate mutations; (C) Unitary pseudogenes are derived without duplication from an ancestral gene; (D) Polymorphic pseudogenes are intact in the reference genome, but have disabling mutations in other non-reference genomes. Red sections stand for the mutations that affect the function of genes.

METHODS OF PSEUDOGENES IDENTIFICATION

Pseudogenes are typically identified by computational pipelines (e.g., HAVANA, PseudoFinder, RetroFinder) and manual curation, relying on sequence similarity to known genes and characteristic disruptions such as the absence of introns, truncations, or an

interrupted open reading frame (ORFs).[5,11-13] However, these sequence-based methods can be constrained by the quality of annotation within a single genome. Therefore, recent comparative genomics approaches provide a complementary strategy to classical identification by detecting gene loss events across species. The pipeline starts by mapping reference genes from one species onto the genomes of other species via genome alignment. It systematically removes false positives arising from: (1) low sequencing quality and assembly gaps; (2) paralogs and processed pseudogenes; (3) alternative splicing isoforms; (4) splice site shifts, alignment ambiguities, intron deletions, and compensating frameshifts; and (5) mutations in the terminal 20% regions of genes (where selection is relaxed). A gene is classified as truly lost (pseudogenized) only if it contains inactivating mutations across multiple exons and retains less than 60% of its intact reading frame. To further validate such loss events, candidate inactivating mutations can be manually validated against Sequence Read Archive (SRA) reads, followed by experimental confirmation via PCR analysis.[14] These methods primarily identify unitary pseudogenes in a phylogenetic context, enabling large-scale discovery of gene losses linked to phenotypic adaptations. Together with traditional pipelines, these tools provide a powerful resource for dissecting pseudogene evolution and functional impacts.

PSEUDOGENES CONTRIBUTE TO ADAPTIVE TRAITS IN VERTEBRATES

Immune adaptation

The vertebrate immune system, integrating both innate and adaptive components, has been shaped by dynamic evolutionary forces including gene birth and death. Across diverse lineages, the inactivation of immune-related genes reflects adaptations to specific pathogen landscapes and ecological niches, revealing that gene decay can be as influential as gene gain in maintaining immune function (Figure 2).

The pseudogenization of Toll-like receptors (TLRs) family members has emerged as a recurrent mechanism of immune adaptation in vertebrates, allowing hosts to fine-tune inflammatory responses in accordance with the dominant pathogen pressures of their ecological niches. A striking example of this adaptive strategy is the convergent loss of the activating receptor *TLR5*, which detects bacterial flagellin. For marine mammals,

aquatic exposure to diverse bacterial pathogens, including *Vibrio*, *Pseudomonas*, and *Streptococcus*,[15-19] poses unique challenges to their innate immune systems.[20] The loss of *TLR5* in marine mammals such as pinnipeds and Yangtze River dolphins (*Lipotes vexillifer*) is consistent with functional evidence from zebrafish, where *tlr5a* knockout attenuates inflammation and improves survival during *Edwardsiella piscicida* infection.[21] Intriguingly, *TLR5* pseudogenization has also occurred in terrestrial mammals (e.g., guinea pig, pangolin) and multiple passerine bird lineages.[22,23] Although the selective drivers in these lineages remain unclear, the loss may similarly mitigate immunopathology. A comparable loss of an activating receptor is seen in penguins. *TLR15*, a fungal product-detecting receptor, has been pseudogenized, which likely restricts immunopathology caused by chronic Aspergillus exposure.[24] In contrast, the inhibitory receptor *TLR10* has been pseudogenized in seven Carnivora species. This loss presumably removes an anti-inflammatory brake, thereby enhancing the proinflammatory responses needed to combat the diverse pathogens encountered through their predatory and scavenging lifestyles.[25]

Beyond TLRs, the pseudogenization of other pattern recognition receptors (PRRs) has similarly emerged as a widespread mechanism of immune adaptation in vertebrates, enabling the recalibration of inflammatory and antiviral responses to distinct ecological pressures. In the RIG-I-like Receptors (RLRs) family, RIG-I, which detects cytosolic viral RNA, has been inactivated independently at least 16 times across approximately 150 bird genomes, nearly always accompanied by loss of its regulator *RIPLET*. Reintroducing a functional RIG-I into chickens exacerbates disease and mortality, indicating that its loss in birds serves to avoid excessive inflammation.[26,27] Among mammals, pangolins display extensive NOD-like Receptors (NLRs) pseudogenization, including the unique loss of *NOD2* and the convergent absence of *NLRC4* and *NAIP* (shared with canine carnivorans), likely to avoid detrimental immune activation by the dense commensal microbiota of their myrmecophagous diet.[28] More broadly, genome-wide analyses across Afrotherian mammals have revealed a burst of gene losses in elephants, hyraxes and manatee, including the inflammasome sensors *AIM2*, *MEFV*, *NLRC4*, *NLRP1* and *NLRP6*, whose pseudogenization may contribute to the repeated evolution of large body size and enhanced cancer resistance.[29]

The major histocompatibility complex (MHC) is the central to adaptive immunity, and the loss or pseudogenization of MHC genes has been linked to both compromised immunity and compensatory evolution. The pseudogenization of MHC II, CD4, and the invariant chain in Atlantic cod (*Gadus morhua*) represents an adaptive evolutionary remodeling, accompanied by compensatory expansions of MHC I and enhanced TLRs diversity.[30] Functional evidence suggests that this loss may have driven the evolution of alternative MHC I-based antigen presentation pathways.[31,32] In marsupials, manual annotation of 384 MHC class II genes across 29 species revealed losses of key components in the Dasyuromorphia order and the Pseudochiridae family, whose loss may reflect a trade-off between the energetic requirements of immunity and reproduction.[33] In baleen whales (Mysticetes), extensive MHC class I pseudogenization alongside the retention of a few ancient loci[34] has been interpreted as a possible consequence of reduced selective constraints on immunity driven by the marine environment and the distinct transmission dynamics of marine pathogens.

The influence of pseudogenization extends beyond receptor molecules to key immune regulators. A primate-specific chimeric processed pseudogene of *IRF5P1* has been identified in apes and humans, whose conservation suggests positive effects on primate evolution and innate immunity.[35] Similarly, a U1 pseudogene, *RNVU1-18*, positively regulates antiviral innate immunity by promoting TRIM25-mediated RIG-I activation, illustrating that pseudogenes can function as positive regulators of key immune signaling pathways.[36] Moreover, the 5S rRNA pseudogene *RNA5SP141* is an endogenous RIG-I ligand required for antiviral innate immunity, and its depletion abrogates RIG-I-mediated responses against Herpes Simplex Virus type 1 (HSV-1).[37] Together, these findings illustrate that pseudogenization acts as a dynamic force in immune evolution, driving both vulnerability and innovation across vertebrate lineages.

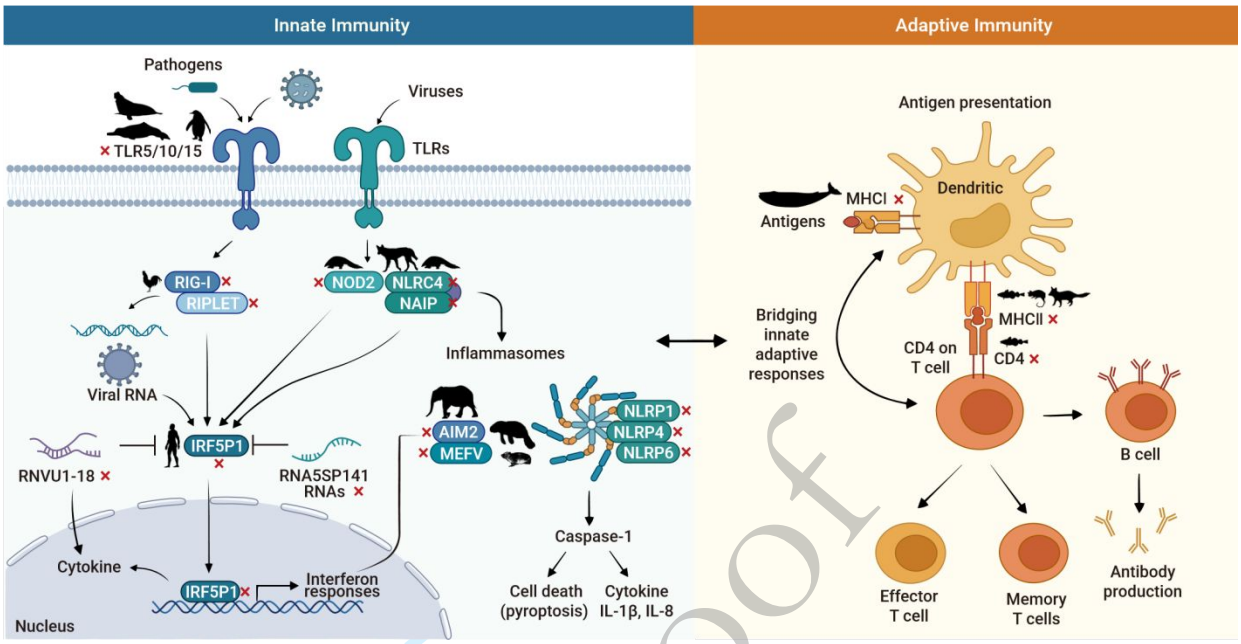


Figure 2. Pseudogenization of genes involved in innate and adaptive immunity in vertebrates This schematic integrates key components of the innate immune system, including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), inflammasome sensors (e.g., *AIM2*, *MEFV*), cytokines, and interferon responses, alongside the adaptive immune system, encompassing antigen presentation (MHC class I/II, CD4), T cell activation, and B cell antibody production. Red crosses mark molecules that have undergone lineage-specific pseudogenization or complete gene loss in one or more vertebrate lineages. Silhouettes of species were taken from Phylopic.org.

Sensory adaptation

Senses, including vision (sight), olfaction (smell), tactition (touch), are essential for animals to predict their environment and reduce uncertainty and risk.[38] The vertebrate senses are an interconnected whole, evolving in response to ecological challenges. The possession of efficient senses and their implications on animal behavior are of significant evolutionary and ecological relevance, and different types of sensory perceptions have played an important role in adaptive radiation, coevolution, and the overall generation of biological diversity (Figure 3). [38]

Visual transduction is initiated by light-activated opsins that trigger transducin signaling, and convergent pseudogenization of both opsin and transducin genes has occurred repeatedly across vertebrate lineages as an adaptation to dim-light environments (Figure 3A). For instance, pseudogenization of the short-wavelength-sensitive opsin gene *SWS1* is prevalent in cave-dwelling bats, revealing the vital role of ecological niche specialization in visual evolution.[39] Caecilians have completely lost *SWS1* and *SWS2* due to their permanently subterranean lifestyle, further supporting that opsin loss represents a convergent adaptive strategy for dark and dim-light habitats.[40] A total of 53 *SWS1* and 12 M/LWS pseudogenes were identified across 220 mammals, including a solenodon (*Solenodon paradoxus*) *SWS1* and right whale (*Eubalaena japonica*), fruit bat (*Eidolon helvum*), and mole vole (*Ellobius lutescens*) M/LWS losses, which benefit dim-light adaptation.[41] In extreme cases, such as naked mole-rats (*Heterocephalus glaber*) and blind mole-rats (*Spalax galili*), multiple genes involved in phototransduction and retinal integrity (*GUCY2F*, *ABCB5*, *RP1L1*, *CRB1*, *ARR3*) have accumulated inactivating mutations, leading to profound eye degeneration adapted to subterranean life.[42]

The pseudogenization of olfactory receptors (OR) genes in vertebrates is overwhelmingly concentrated in species that experienced invasion into new environments (e.g., aquatic habitats and highlands), suggesting that ecological shifts have driven the degeneration of OR genes (Figure 3B). For example, the common carp (*Cyprinus carpio*) exhibits a reduced OR gene repertoire with a high proportion of pseudogenes, suggesting increased dependence on taste and vision during foraging.[43] In odontocetes, eight genes encoding key proteins of the olfactory signal transduction pathway (e.g., *GNAL*, *ADCY3*, *CNGA2*, *CNGA4*, *CNGB1*, *SLC8A1*) have all undergone inactivating mutations, which may be closely related to the evolution of advanced echolocation.[44] Furthermore, a study systematically analyzing the OR gene repertoires of 25 carnivore species found that marine carnivores (e.g., sea lions and seals) have experienced significant degeneration in OR gene number and pseudogene proportion, consistent with reduced olfactory reliance in the aquatic environment.[45] Convergent reduction of olfaction has also been documented in terrestrial mammals adapted to highlands. A comparative genomic analysis revealed that high altitude lineages have convergently lost function in their OR

repertoires. This general relaxation of constraint on OR genes at altitude may arise from reduced odorant diversity in high-altitude environments or reduced effectiveness of mammalian olfactory physiology in thin, dry, or cold air.[46]

In marine mammals, the skin has undergone profound structural remodeling to meet the demands of aquatic life (Figure 3C). The epidermis is thickened for enhanced resistance and homeostasis, yet its upper layer remains incompletely cornified, resulting in smooth skin that lacks typical mammalian features such as pelage, sebaceous glands, and sweat glands.[47,48] This morphological transition is underpinned by extensive pseudogenization of skin-related genes, which reduces energy expenditure and confers selective advantages in aquatic environments. Convergent gene loss has been documented across multiple marine and semi-aquatic lineages. Marine mammals exhibit inactivating mutations in keratin genes (*K1*, *K2*, *K9*, *K10*), keratinization regulators (*SERPINA12*, *SLURP1*), sebaceous gland genes (*MOGAT3*, *DGAT2*, *AWAT1*), and hair follicle development genes (*FGF22*).[47,49] In dugongs, RNA-seq analysis confirmed the loss of additional skin-related genes (*ALOX15*, *AWAT2*, *LYG2*), many of which are also pseudogenized in cetaceans, indicating parallel evolutionary trajectories.[50] Further extending this pattern, Springer et al. (2021) identified ten skin-associated genes (e.g., *ALOX15*, *AWAT1*) with inactivating mutations shared between hippopotamuses and cetaceans.

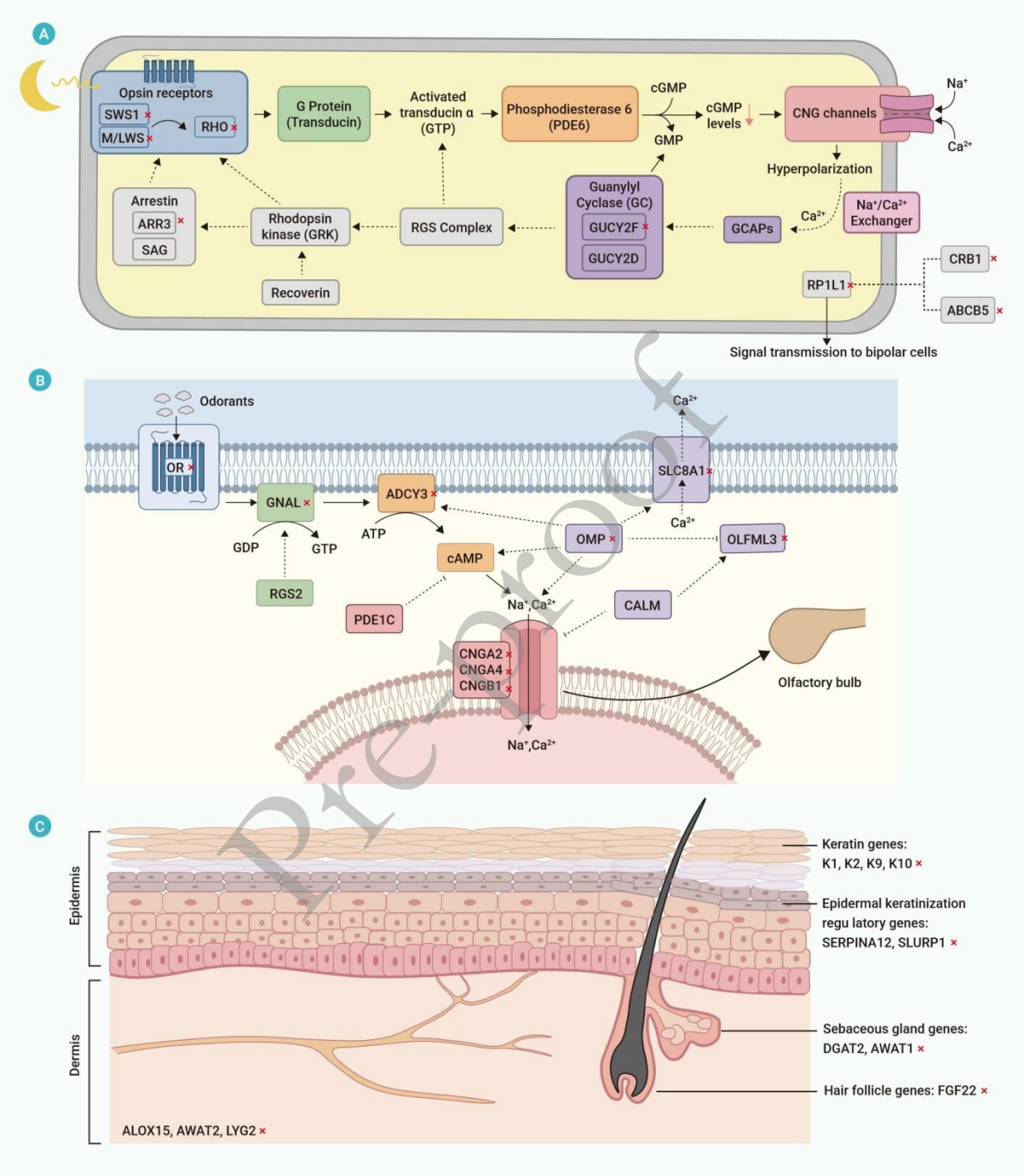


Figure 3. Pseudogenization of sensory-related genes in habitat-adapted vertebrates This schematic summarizes genes involved in opsin-transducin (A), olfactory receptor/signal transmission (B), and skin morphogenesis (C) that have acquired inactivating mutations in vertebrates. Pseudogenes are marked by a red "×".

These gene losses reflect morphological and sensory remodeling during adaptation to diverse environments.

Adaptation to dietary changes

The morphology, ecological role, and behavior of vertebrates are largely determined by their feeding ecology, making dietary adaptation a major source of natural selection. Dietary adaptation is frequently accompanied by convergent pseudogenization of tooth development, taste receptor, and digestive enzyme genes, which facilitate dietary transitions and nutritional adaptation (Figure 4).[51,52]

A large number of tooth formation and enamel development genes exhibit widespread convergent pseudogenization across vertebrates that have evolved tooth simplification or filter-feeding habits. For example, as tongue prehension specialists, true toads (Bufonidae) transport small prey directly into the esophagus, eliminating functional need for teeth. This relaxed selective constraint on dental structures is mirrored by the shared pseudogenization of the enamel gene *AMEL* in bufonids.[53,54] Genomic analysis of xenarthrans (anteaters, sloths, and armadillos) reveals pseudogenization of core dental genes including *ENAM*, *AMELX*, *MMP20*, and *DSPP*, indicating parallel dental degeneration among closely related species.[55] Additionally, among cetaceans, baleen whales exhibit inactivating mutations or deletions in genes critical for enamel formation (*ODAM*, *ACPT*), underscoring their complete reliance on filter feeding rather than mastication.[56,57] These studies illustrate that tooth-related gene loss is a recurring evolutionary strategy accompanying transitions in both diet and feeding mode.

Taste governs feeding behavior, with umami and sweet sensations mediated by taste 1 receptors (TAS1Rs) and bitter perception by taste 2 receptors (TAS2Rs).[58] Pseudogenization of taste genes can reduce or eliminate sensitivity to specific tastants, enabling animals to exploit novel food resources.[59] For example, felids, as obligate carnivores, have lost sweet perception due to pseudogenization of *TAS1R2*. In contrast, otariids (sea lions) swallow prey whole without chewing and have lost both sweet and umami perception through simultaneous pseudogenization of *TAS1R1* and *TAS1R3*.[60] Lu et al. found that the common vampire bat (*Desmodus rotundus*) has lost a large number of bitter taste receptor genes TAS2Rs, consistent with its dietary shift from insectivory to

obligate sanguivory.[61] Giant pandas (*Ailuropoda melanoleuca*) exhibit extensive frameshift mutations in *TAS1R1* (umami) and multiple TAS2Rs genes, likely facilitating their transition to an almost exclusive bamboo diet.[59,62] These cases demonstrate that taste gene degradation does not represent random functional decay, but a targeted adaptive adjustment of sensory taste systems, which optimizes feeding decision-making to fit long-term stable dietary characteristics.

Food choices are constrained by digestive capacity, with digestive enzymes playing a central role in nutrient extraction.[63,64] Adaptation to a carnivorous or protein-rich diet often involves the loss of genes related to plant digestion or carbohydrate metabolism. For instance, cetaceans, which transitioned from herbivorous to carnivorous diets, exhibit frameshift mutations or premature stop codons in multiple pancreatic protease genes (*CPA2*, *PRSS2*, *CTRL*, *CELA2A*).[65] The high proportion of *RNASE1* pseudogenes in non-herbivorous lineages suggests that losing this plant-RNA-digesting enzyme helps avoid unnecessary synthesis costs and conserve metabolic resources.[66] Hecker et al. further showed that carnivores lost *INSL5-RXFP4* (appetite/glucose regulation), *NR1I3* (xenobiotic receptor), and *NOX1* (gut immunity), each loss aligning with specific dietary adaptations in feeding pattern, nutrient composition, or microbiome diversity. Conversely, adaptation to an herbivorous or low-fat diet is frequently accompanied by the loss of genes involved in fat digestion and non-plant nutrient processing. For instance, herbivorous mammals repeatedly lost *PNLIPRP1* (fat digestion inhibitor) and *SYCN* (zymogen secretion facilitator).[67] In addition, *TREH* encodes an enzyme that digests trehalose, the primary blood sugar in insects. This gene has been repeatedly pseudogenized in bats and other mammalian lineages that shifted from insectivory to non-insectivorous diets (e.g., frugivory, nectarivory, sanguivory). Enzymatic assays confirmed that trehalase activity is significantly reduced or lost in non-insectivorous bats.[68] These findings highlight that digestive enzyme gene loss is not random but tightly coupled with dietary ecology, enabling metabolic optimization for specific food sources.

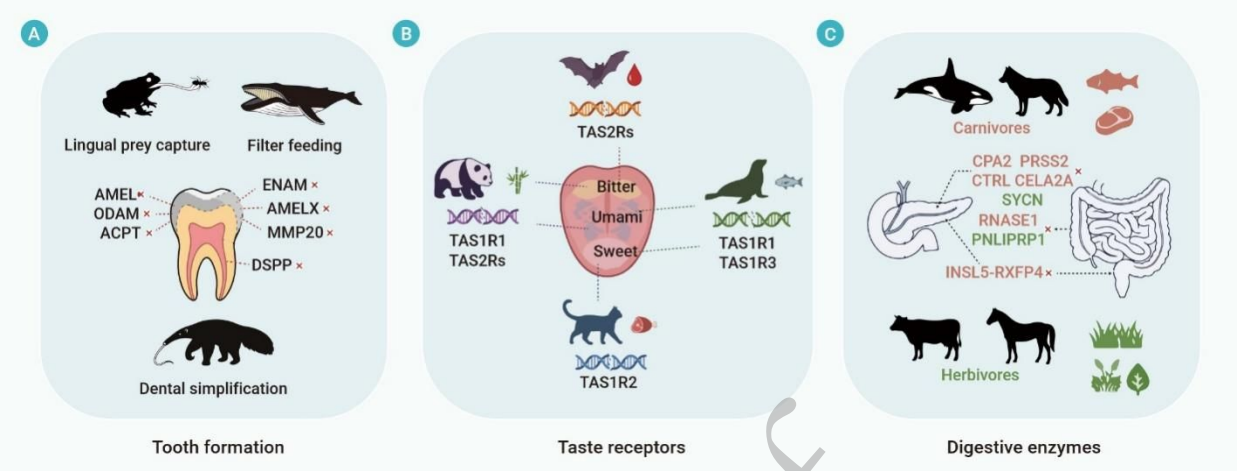


Figure 4. Pseudogenization of dietary-related genes in vertebrates This schematic summarizes inactivated genes involved in tooth formation (A), taste perception (B), and digestion (C) in herbivores and carnivores. Pseudogenes are marked by a red "×". In the digestive enzyme panel, genes in red indicate pseudogenization in carnivores, while genes in green indicate pseudogenization in herbivores. Gene losses correlate with feeding adaptations such as filter feeding, lingual prey capture, and dental simplification. Silhouettes of species were taken from Phylopic.org.

Sex determination

Sex determination mechanisms in vertebrates exhibit remarkable diversity, yet the genetic factors governing this process remain poorly characterized across many taxa, including reptiles, birds, and mammals.[22,69] Accumulating evidence suggests that pseudogenization plays an active role in shaping sex determination and reproductive evolution. In the frog (*Rana rugosa*), the *CYP17A1* gene contributes to male determination, while its pseudogene *CYP17A1P* shows low expression in both sexes, indicating that pseudogenization rendered this copy inert while the ancestral gene retained its male-determining function.[70] A more striking case is found in African clawed frogs (*Xenopus laevis*), where the female-determining gene *DM-W* originated from the male-associated gene *DMRT1* following pseudogenization of the latter, suggesting that gene degradation can paradoxically generate new sex-determining loci.[71] Similarly, in Percidae fishes, the Y-linked sex-determining gene *amhr2bY* has been independently lost in *Perca fluviatilis* and *Sander vitreus*, while it has been amplified in *Sander lucioperca*.

The loss of *amhr2bY* in *P. fluviatilis* is associated with the emergence of a new XX/XY sex-determination system, indicating that the pseudogenization of an ancestral sex-determining gene can drive the turnover of sex determination mechanisms.[72]

Beyond its direct role in altering individual sex-determining loci, pseudogenization is a general feature of sex chromosome evolution.[73] This is exemplified by the human Y chromosome, where the pseudogenization and complete loss of X-linked gametologs illustrate the extensive gene decay characterizing this chromosome.[74] In marsupials, sequencing of Y chromosome-specific BAC clones from the tammar wallaby (*Macropus eugenii*) reveals multiple pseudogenes with testis-specific expression, indicating that the Y chromosome serves as a reservoir for evolving and decaying reproductive genes.[75] Likewise, a comparative analysis of Y chromosomes across 29 primate species reveals markedly different rates of gene loss among lineages over 80 million years of evolution, underscoring the role of gene degradation in sex chromosome evolution.[76] Beyond the Y chromosome, the sperm-egg adhesion gene *ZP3R* has undergone recurrent independent pseudogenization in apes and monkeys. Its degeneration is linked to relaxed sexual selection, implying a selective cost to maintaining fertilization genes that are no longer essential under reduced sperm competition.[77]

Metabolic adaptation

Pseudogenes have emerged as important drivers of metabolic adaptation, particularly when shifts in dietary composition and energy demand impose new physiological requirements. Hummingbirds perform energy-intensive hovering flight and possess an inactivated *FBP2*, a muscle-specific gluconeogenic enzyme that counteract glycolysis. Knockdown of *FBP2* in an avian myoblast cell line increases glycolytic flux, mitochondrial biogenesis, and respiration, hallmarks of hummingbird flight muscle that underpin the highest mass-specific metabolic rate among vertebrates.[78] Fruit bats feed predominantly on sugar-rich nectar with minimal fat and protein and show a lineage-specific loss of *FFAR3*, an inhibitor of insulin secretion. Consistent with this, *Ffar3* knockout mice exhibit enhanced glucose-stimulated insulin release and improved glucose tolerance, mirroring the elevated insulin levels observed in frugivorous bats and facilitating efficient

carbohydrate processing on a sugar-based diet.[14] In cetaceans, the pseudogenization of *ADRB3* and *UCP1*, master regulators of adipose tissue lipolysis and non-shivering thermogenesis, has promoted blubber thickening. Functional assays confirm that this pseudogenization leads to reduced thermogenesis and suppressed lipolysis, causing lipid accumulation and thereby driving blubber thickening.[79,80] Similar to these examples, the giant panda has acquired a lineage-specific loss-of-function mutation in the *DUOX2* gene, which attenuates thyroid hormone synthesis and drives a profound reduction in energy expenditure—an adaptation to its low-energy bamboo diet.[81,82] These findings collectively suggest that pseudogenization actively rewires core metabolic pathways in response to diverse ecological challenges.

PSEUDOGENES FOR HUMAN DISEASE

Unlike their counterparts in many other vertebrates, which are more commonly documented as having undergone complete functional loss, numerous human pseudogenes are now being identified that retain partial biological activity as duplicated copies of functional genes. These "genomic fossils" can influence gene expression and regulatory networks through diverse mechanisms—acting as decoys for regulatory molecules, modulating mRNA stability, or titrating microRNA activity. Elucidating how pseudogenes contribute to disease states not only deepens our understanding of gene regulation but also opens new avenues for diagnostic and therapeutic innovation (Figure 5).

Building upon traditional classification, emerging evidence has further reclassified pseudogenes from non-functional genomic fossils into three major mechanistic categories according to their mode of action. (1) Non-coding mechanisms: Many pseudogenes are transcribed into long non-coding RNAs (lncRNAs) that function as competing endogenous RNAs (ceRNAs). These pseudogene-derived lncRNAs competitively bind to microRNAs, thereby relieving miRNA-mediated repression of their target messenger RNAs (mRNAs). This mechanism is extensively documented in cancer stem cell regulation and tumorigenesis.[83-85] (2) Coding-dependent mechanisms: Despite their disabling mutations, some pseudogenes retain or regain the capacity to be translated. Long-read sequencing studies have identified pseudogene transcripts with

intact ORFs that are translated into truncated or neomorphic peptides in human cells.[86,87] (3) Functional DNA sequences: Pseudogenes can also exert regulatory functions directly at the DNA level without being transcribed, functioning as cis-regulatory elements or participating in chromatin modulation.[87]

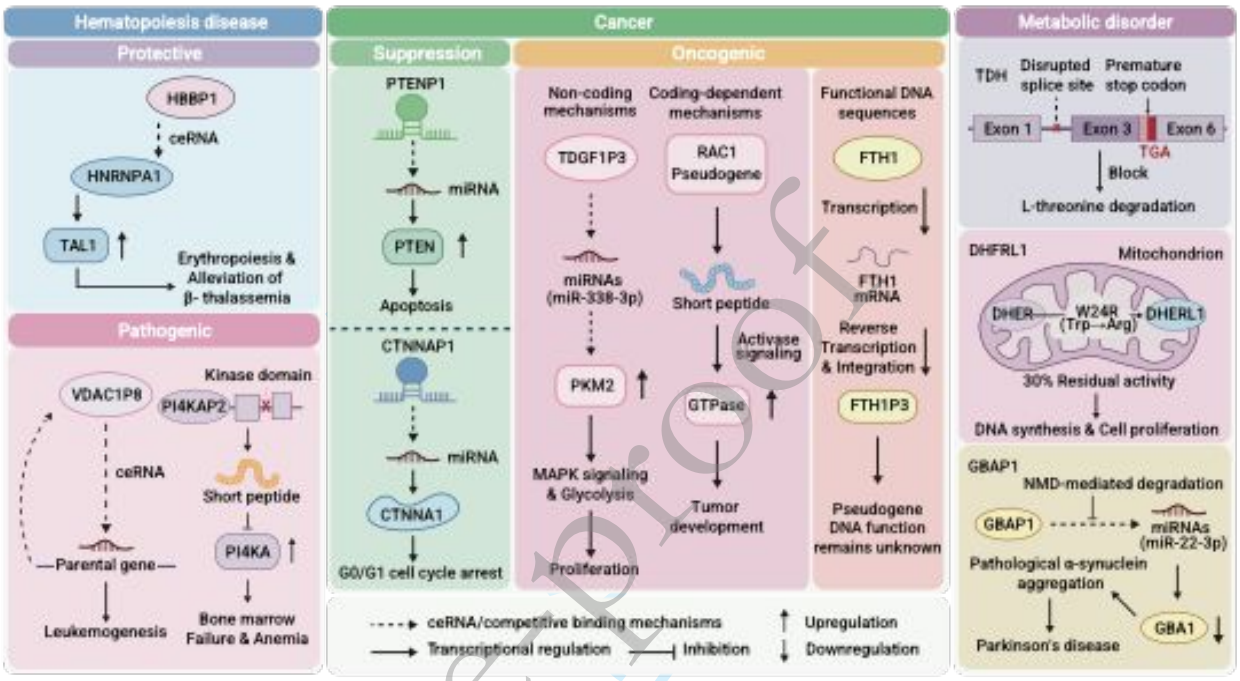


Figure 5. Pseudogenes involved in human diseases The diagram summarizes key pseudogenes implicated in three major disease categories: hematopoiesis, metabolic disorders, and cancer. These findings illustrate the functional duality of pseudogenes in human pathology, where they act as either protective regulators or pathogenic drivers.

Cancer

Dysregulation of pseudogenes can affect crucial processes such as cell proliferation, apoptosis, and metastasis. Transcripts of most tumor-associated pseudogenes can act as ceRNA to maintain the expression of their parental genes by competing for the binding of some identical microRNA molecules, thereby promoting or inhibiting tumor development.[88] For example, *PTENP1* is a lncRNA that is thought to be a pseudogene of the *PTEN* tumor suppressor gene. Downregulation of miRNAs by *PTENP1* inhibits the proliferation, migration, and aggressiveness of cancer cells.[89] *CTNNAP1* plays a key role in the regulation of its homologous gene *CTNNA1* by competing miR-141.

Overexpression of *CTNNAP1* or *CTNNA1* significantly inhibits cell proliferation and tumor growth *in vitro* and *in vivo* by inducing G0/G1 cell cycle arrest. For example, subcutaneous injection of *CTNNAP1* into mice significantly inhibited the development of colorectal cancer tumors.[90]

In contrast, some pseudogenes can contribute to cancer development through common signaling pathways. Non-coding mechanisms represent a major pathway through which pseudogenes exert their oncogenic effects. For instance, *TDGF1P3* acts as a ceRNA by sponging miR-338-3p, blocking its interaction with the target *PKM2* and upregulating *PKM2* expression, thereby promoting colorectal cancer (CRC) cell proliferation and invasion.[91] *FTH1P3* is an intron-less pseudogene derived from retrotransposition of *FTH1* processed mRNA and expresses a ceRNA. This pseudogene is up-regulated in several human cancers, where its increased expression is associated with proliferation, migration, invasion, promotion of epithelial-mesenchymal transition, and drug resistance.[92] Coding-dependent mechanisms also contribute to pseudogene-mediated tumorigenesis. The *RAC1* pseudogene is an intron-free pseudogene containing 579 nucleotides that translates only 46 amino acids. Higher frequencies of *RAC1* pseudogene overexpression are detected in human brain tumors. *RAC1* activates GTP and upregulates the signaling pathway triggered by GTPase, thereby promoting tumor development.[93] Functional DNA sequences represent a third category of pseudogene action, although their roles remain less defined. Several *FTH1* family pseudogenes (e.g., *FTH1P1-12*) were derived from retrotransposition of *FTH1* processed mRNA and exhibit characteristics of processed pseudogenes.[92] However, their potential functions at the DNA level remain to be elucidated.

Hematopoiesis disease

Pseudogenes actively participate in hematopoietic regulation, with both protective and pathogenic roles documented. Several pseudogenes function as ceRNAs or lncRNAs to safeguard normal blood development. *HBBP1*, a duplicated pseudogene essential for human erythropoiesis, alleviates β-thalassemia by competitively binding *HNRNPA1* and upregulating the transcription factor *TAL1*.[94] Similarly, *BMI1P1* acts as a ceRNA to inhibit *BMI1*, blocking the reprogramming of myeloid progenitors into leukemia stem cells and

maintaining normal hematopoiesis.[95] *TPTEP1* is an anti-tumor lncRNA in acute myeloid leukemia (AML), suppressing JNK signaling and inhibiting leukemic cell proliferation.[96] Conversely, *PI4KAP2* acts as an antagonistic regulator of *PI4KA* lacking kinase domains, it contributes to anemia, bone marrow failure, and leukemogenesis.[97] *VDAC1P8* is a processed pseudogene of *VDAC1* that is overexpressed in acute myeloid leukemia and may function as a ceRNA to regulate its parental gene expression, thereby contributing to leukemogenesis.[98]

Metabolic disorder

Metabolism is a crucial pathway for all living organisms, essential for obtaining, absorbing, and utilizing nutrients. Beyond intact enzymes, pseudogenization or decayed copies of metabolic genes often retain unexpected functions, either residual catalytic or novel non-catalytic. Such functions can significantly shape metabolic outcomes and disease vulnerability. For instance, the GCase (encoded by *GBA1*) possesses a pseudogene copy, *GBAP1*, whose transcript acts as a ceRNA to positively regulate *GBA1* expression by sponging miR-22-3p. However, excessive NMD-mediated degradation of *GBAP1* may diminish its ceRNA effect, promoting glucosylceramide accumulation and pathological α-synuclein aggregation, thereby contributing to Parkinson's disease.[99,100] *RPL7P1*, a ribosomal protein pseudogene, has been shown to contribute to diabetes coexisting with infections (DCI). It acts as a ceRNA to sponge miR-144-3p and regulates cuproptosis (a novel form of cell death) via m6A modification, thereby influencing both metabolic and immune pathways.[101] *TDH* is a typical expressed pseudogene that loses the splice receptor site before exon 6, and the codon arginine-214 (CGA) mutates to a stop codon (TGA). *TDH* plays a catalytic role in the degradation of L-threonine. Thus its dysfunction leads to blockage of the L-threonine degradation pathway.[102] *DHFRL1*, located in mitochondria, has no intron structure. The conserved catalytic tryptophan (W24) in *DHFR* becomes arginine in *DHFRL1*, resulting in a 30% reduction in enzyme activity. However, *DHFRL1* is still able to promote de novo DNA synthesis and cell proliferation, suggesting that this pseudogene may supplement mitochondrial folate metabolism demands.[103]

PERSPECTIVES

The growing recognition of pseudogenes as dynamic genomic elements rather than passive evolutionary relics has opened new avenues for understanding the molecular basis of adaptive evolution and human disease. Future research should move beyond cataloging pseudogenization events toward elucidating the mechanistic links between gene loss and phenotypic innovation. Integrating comparative genomics with functional assays across diverse vertebrate lineages will be essential to determine how pseudogene formation contributes to lineage-specific adaptations. In addition, base editing can be deployed to precisely introduce or revert stop codons in these cryptic ORFs, thereby providing a direct test of their causal contribution to lineage-specific adaptive traits. The convergent pseudogenization patterns observed in phylogenetically distant species facing similar selective pressures, offer powerful models for investigating the predictability of evolutionary trajectories. Moreover, the emerging roles of pseudogene-derived regulatory RNAs, including lncRNAs and ceRNAs, highlight the potential for "non-functional" copies to acquire novel functions that fine-tune gene expression networks in response to environmental challenges. However, a key unresolved question is whether pseudogene-derived regulatory RNAs, which are well characterized in human disease, also contribute to adaptive evolution in vertebrates. Progress has been limited because adaptation research has centered on unitary pseudogenes, and exploring such regulatory roles remains severely constrained by the experimental intractability of diverse non-model vertebrate lineages. Advances in long-read sequencing, CRISPR-based functional screening, and long-read RNA-seq data will improve the discovery of novel pseudogenes and the correction of false-positive annotations. These technologies will also enable precise dissection of how pseudogene transcripts modulate cellular processes during development, physiology, and stress responses. From an evolutionary developmental biology (Evo-Devo) perspective, exploring how pseudogenization of key developmental genes shapes morphological innovation will provide deeper insights into the genetic basis of adaptive traits. Ultimately, integrating pseudogene dynamics into the framework of adaptive evolution will not only illuminate the evolutionary forces shaping vertebrate genomes but also reveal novel targets for understanding human diseases arising from dysregulation of these once-overlooked genomic elements.

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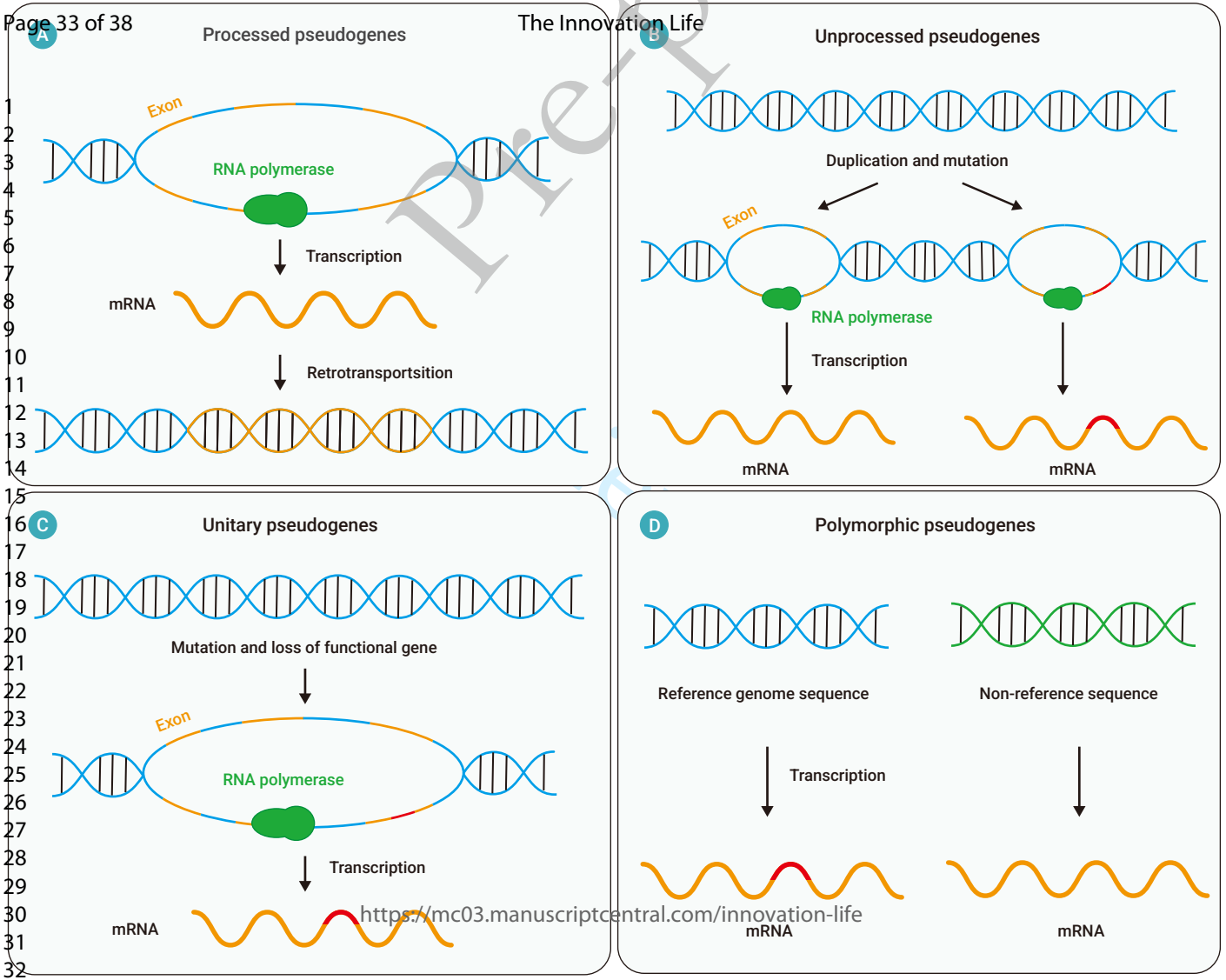
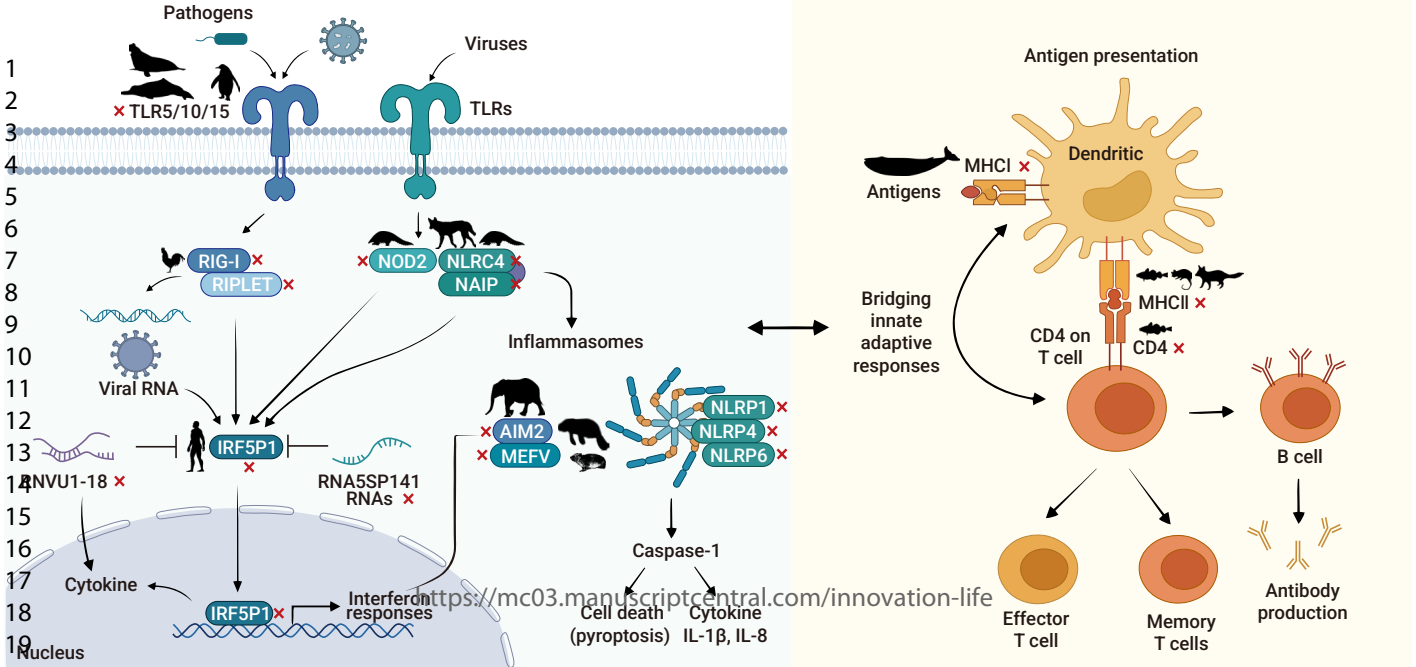
AUTHOR CONTRIBUTIONS

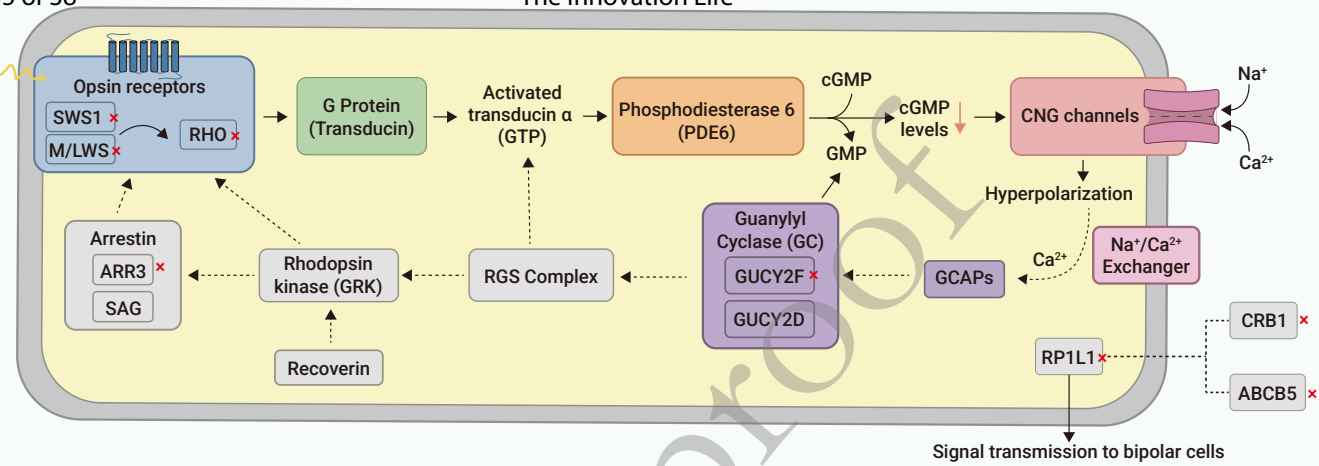
Zhihong Jin and Xiangman Meng were primarily responsible for the manuscript writing and figure preparation. Liang Zhao, Lydia Mukanhaire, Qiang Zhou, and Qianqi Liu contributed to data collection, literature review, and provided support for the development of research ideas. Lin Zhang and Ran Tian as corresponding authors, conceived and designed the overall review framework, supervised the review process, and revised the manuscript. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.







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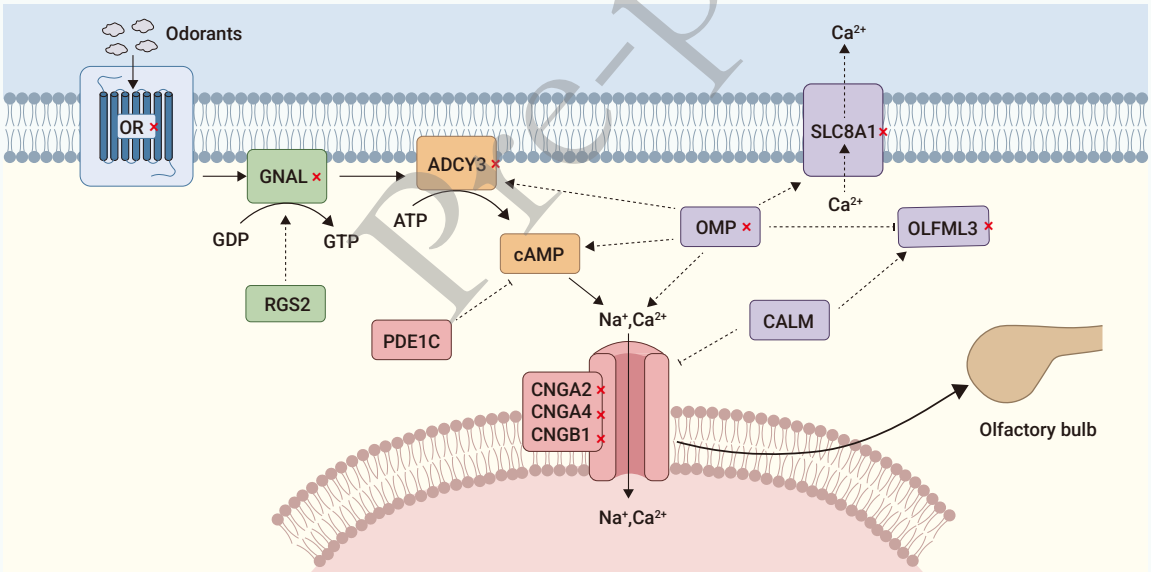

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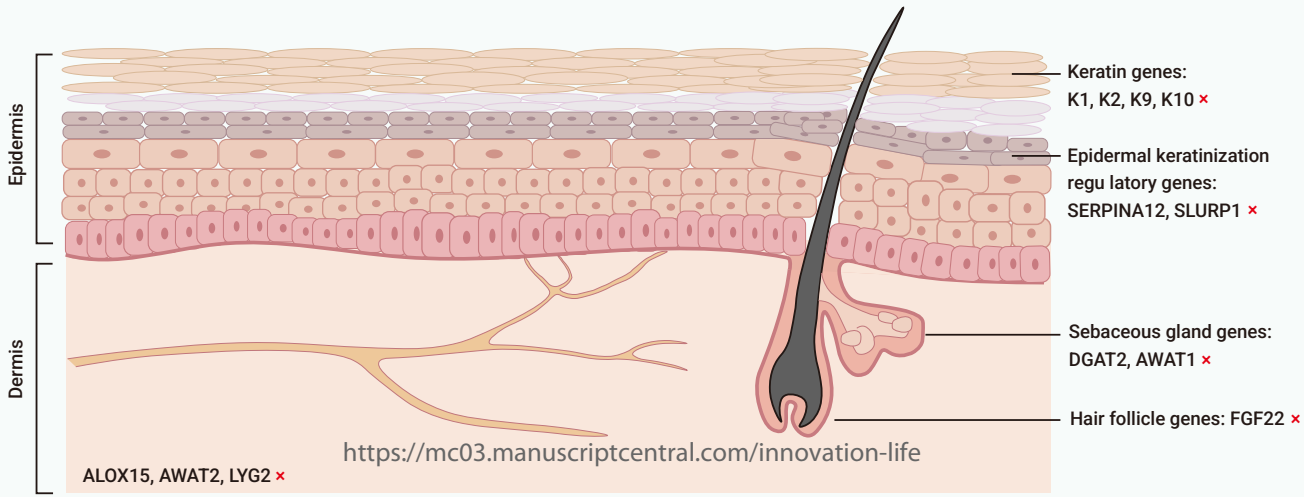
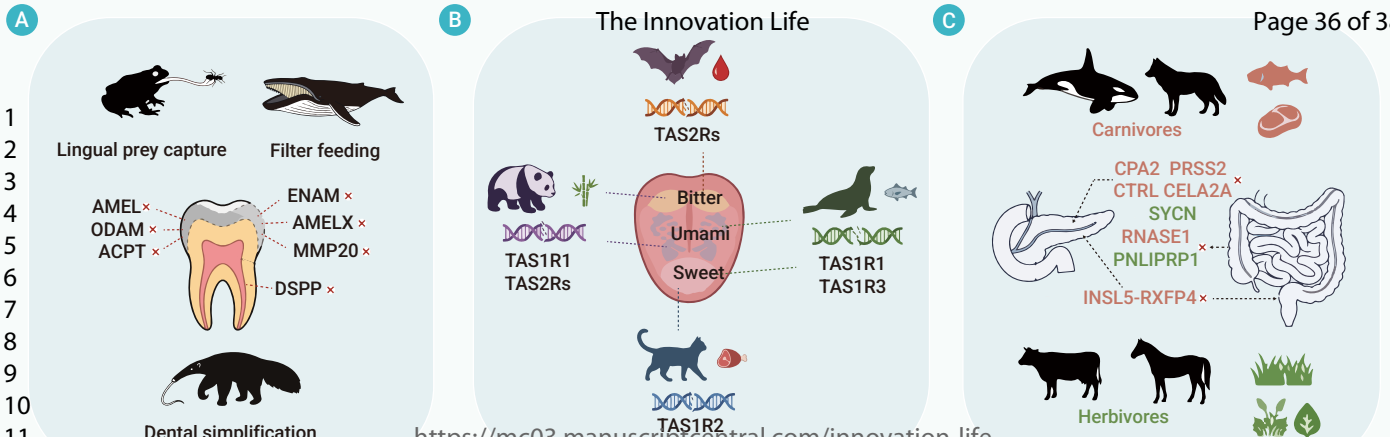
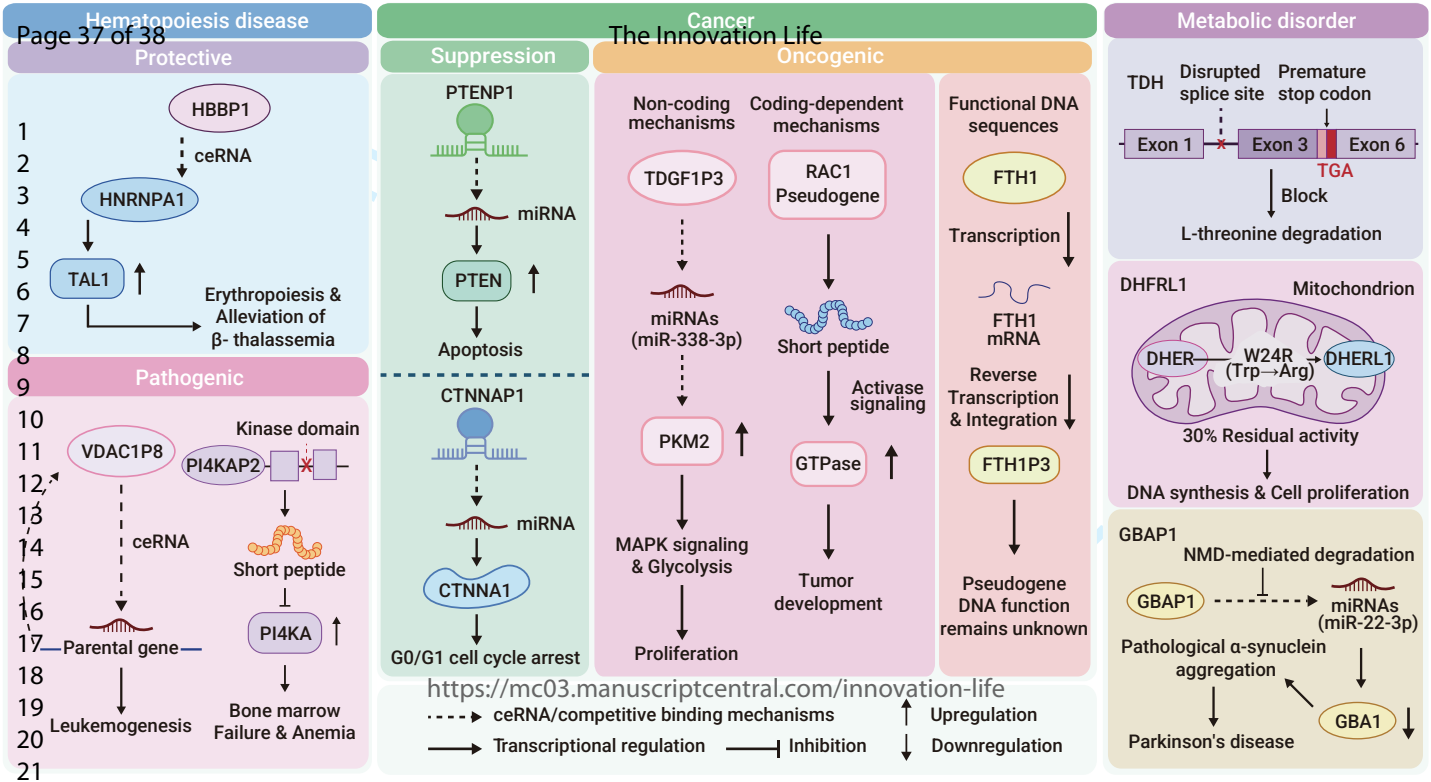


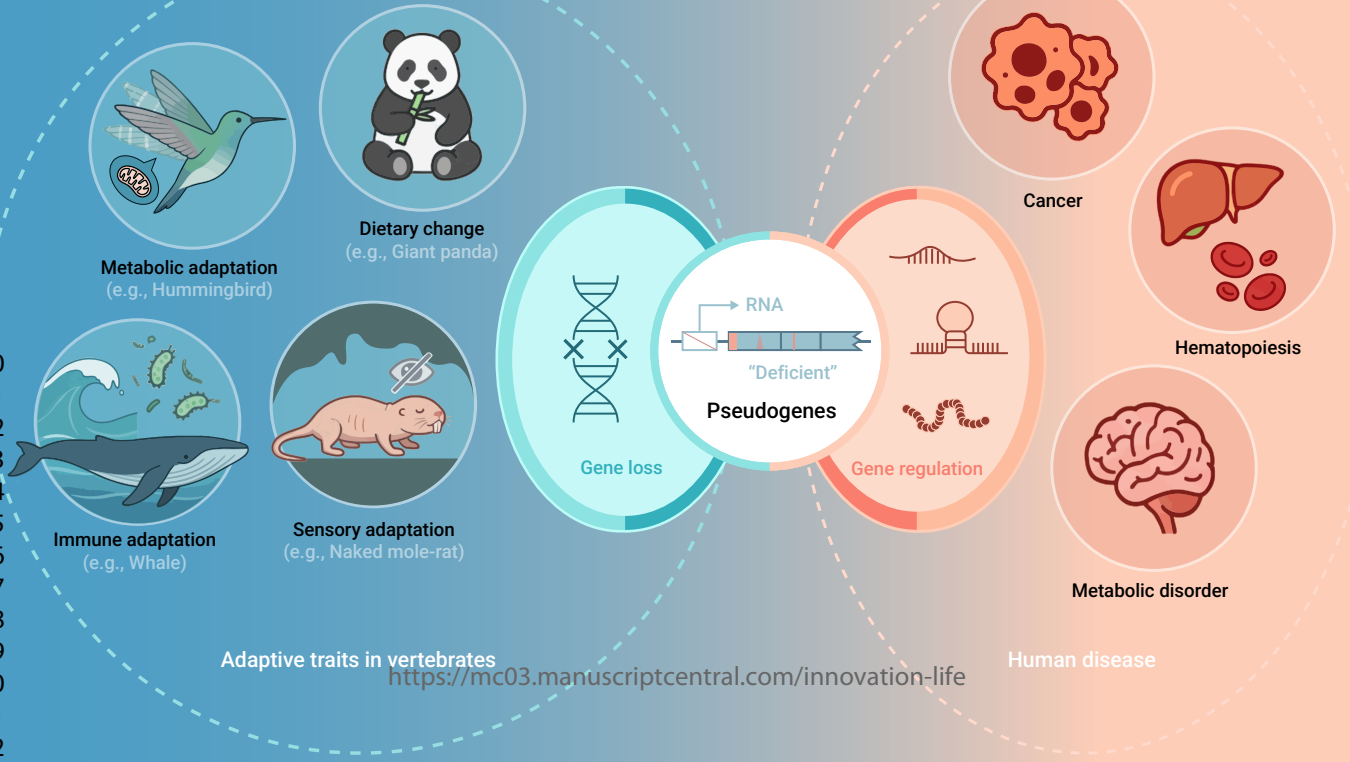


Tooth formation

Taste receptors

Digestive enzymes


Hematopoiesis disease
Protective
HBBP1
ceRNA
HNRNPA1
TAL1
Erythropoiesis & Alleviation of β- thalassemia
Pathogenic
VDAC1P8
ceRNA
Parental gene
Leukemogenesis
Kinase domain
PI4KAP2
Short peptide
PI4KA
Bone marrow Failure & Anemia
Cancer
Suppression
PTENP1
miRNA
PTEN
Apoptosis
CTNNAP1
miRNA
CTNNA1
G0/G1 cell cycle arrest
Oncogenic
Non-coding mechanisms
Coding-dependent mechanisms
TDGF1P3
miRNAs (miR-338-3p)
PKM2
MAPK signaling & Glycolysis
Proliferation
RAC1 Pseudogene
Short peptide
Activase signaling
GTPase
Tumor development
Functional DNA sequences
FTH1
Transcription
FTH1 mRNA
Reverse Transcription & Integration
FTH1P3
Pseudogene DNA function remains unknown
Metabolic disorder
TDH
Disrupted splice site
Premature stop codon
Exon 1
Exon 3
Exon 6
TGA
Block
L-threonine degradation
DHFRL1
Mitochondrion
DHER
W24R (Trp→Arg)
DHERL1
30% Residual activity
DNA synthesis & Cell proliferation
GBAP1
NMD-mediated degradation
GBAP1
miRNAs (miR-22-3p)
Pathological α-synuclein aggregation
GBA1
Parkinson's disease
ceRNA/competitive binding mechanisms
Transcriptional regulation
Inhibition
Upregulation
Downregulation


Metabolic adaptation
(e.g., Hummingbird)
Dietary change
(e.g., Giant panda)
Immune adaptation
(e.g., Whale)
Sensory adaptation
(e.g., Naked mole-rat)
Gene loss
RNA
"Deficient"
Pseudogenes
Gene regulation
Cancer
Hematopoiesis
Metabolic disorder
Adaptive traits in vertebrates
Human disease