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Special Topic

Diversity and evolution of MicroRNA gene clusters

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microRNA (miRNA) gene clusters are a group of miRNA genes clustered within a proximal distance on a chromosome. Although a large number of miRNA clusters have been uncovered in animal and plant genomes, the functional consequences of this arrangement are still poorly understood. Located in a polycistron, the coexpressed miRNA clusters are pivotal in coordinately regulating multiple processes, including embryonic development, cell cycles and cell differentiation. In this review, based on recent progress, we discuss the genomic diversity of miRNA gene clusters, the coordination of expression and function of the clustered miRNAs, and the evolutionarily adaptive processes with gain and loss of the clustering miRNA genes mediated by duplication and transposition events.

microRNA gene clusters, gene duplication, adaptive evolution, transposition

MicroRNAs (miRNAs) are a class of endogenously small noncoding RNAs about 20—25 nucleotides (nt) in length, which negatively regulate expression of target genes at the post-transcriptional level[1]. In animals, miRNA genes are firstly transcribed by RNA polymerase II[2] or RNA polymerase III[3] to generate primary miRNA transcripts (pri-miRNAs)[4], then processed by the Drosha (a member of the RNase III family) and the DGCR8 (also known as Pasha) complex[5] to produce the hairpin precursors of 70—90 nt (pre-miRNAs). Mediated by Exportin 5[6], the pre-miRNAs are then exported into the cytoplasm, where another RNase III enzyme, dicer incises pre-miRNAs into double-stranded mature miRNA with 2 nt 3′ overhangs. Finally, one strand is rapidly degraded (except for some miRNAs whose two strands are functional, such as miR-151), the remaining strand imperfectly matches the 3′ untranslated regions (3′UTR) of mRNA targets under the guidance of the RNA-induced silencing complex (RISC), resulting in suppression, destabilization or degradation of mRNAs, which depends on the degree of sequence complementarities[4]. Many studies have indicated[7–10] that complementary base-pairing of the seed region (2nd-8th nt of mature miRNA) with 3′UTR is the key for determining the extent of the inhibition of the targets.

Recently, a large number of studies have suggested that miRNAs are critical in the regulation of spatiotemporal gene expression during development[11,12], cell differentiation[13,14], viral defence[15], and control of stress[16]. Dysfunction of this molecular switch (miRNA) will lead to onset of many diseases, such as cancer[17,18], neurodegenerative diseases[19] and cardiac disease[20].

Large-scale surveys of noncoding DNA found that many miRNA genes tend to form clusters[21,22], rather than being randomly distributed across chromosomes. Functional roles of the clustering of miRNAs, their origins and evolutionary processes remain unclear. In this review, based on the recent findings of miRNA clusters, we discuss the genomic diversity of miRNA gene clusters, the coordination of expression and function of the clustered miRNAs and the evolutionary pattern of clustering. Meanwhile, by case studies, we will explain the importance of the gain-and-loss of miRNA clusters in modulating gene expression network and speciation.

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1 The diversity of miRNA clusters

Gene clusters are usually composed of two or more related genes which are adjacently located on a chromosome[23]. Although the clustered genes are not necessarily identical, they usually share sequence similarity. Recently, Lai et al.[22] found that fruit fly miRNA genes are often clustered together. The initial studies indicated that about 50% of total miRNA genes throughout the *Drosophila* genome were clustered, whereas only few miRNA genes might be clustered within the human genome[4]. However, with the increasing number of identified miRNAs, it is suggested that miRNAs are more likely to cluster together than previously estimated. For example, provided that the pairwise chromosomal distances at 3 kb between two miRNA genes is the maximum inter-miRNA distance (MID), Altuvia and colleagues[24] discovered 31 miRNA clusters containing 76 miRNAs, accounting for 37% of all the analyzed 207 human miRNAs from the Sanger miRBase (release 4.0). If the MID is limited to 1 kb, the results from the genome-wide study of Megraw et al.[25] of four species (human, mouse, rat and chicken) showed over 30% of miRNA genes are clustered. When the MID is expanded to 50 kb, nearly half of the miRNA genes are apt to cluster. Consequently, the number of clustering miRNAs is significantly higher than expected, suggesting the clustering pattern of miRNAs may have common *cis*-regulatory elements. Here, we list the numbers of miRNA precursor clusters in three mammalian genomes-*Homo sapiens*, *Mus musculus*, *Rattus norvegicus* (Figure 1) taken from the smiRNAdb database (http://www.mirz.unibas.ch/smiRNAdb/cgi/smiRNAdb?page=home), in which the MID is defined as 50 kb.

In addition, based on bioinformatic prediction, Tang and colleagues[26] discovered that the *Xenopus* miRNA genes were predominantly located within introns and almost 50% of total discovered miRNA species were prone to clustering. Intriguingly, part of the *Xenopus* miRNA clusters were found to have duplicated clusters. For example, designated as "Oncomirs" (miRNAs function as tumor suppressors and oncogenes) in the human genome, the miR-17/miR-92 cluster[17,27] formed a duplicated cluster in *Xenopus*.

As shown above, it is suggested that miRNA clusters are largely present in metazoan genomes, exhibiting the diversity of their distribution. If members of miRNA cluster are homologous, this type of miRNA cluster forms the miRNA gene family, further expanding the diversity of miRNA clusters. For example, a miRNA cluster of seven components is adjacently clustered within a region of 800 bp on the second chromosome of *Caenorhabditis elegans* (*C. elegans*), representing a paralogous miRNA cluster[28] (Figure 2). Although many miRNA clusters have been identified mainly through algorithmic prediction, the functional roles of the non-random distribution are still unclear.

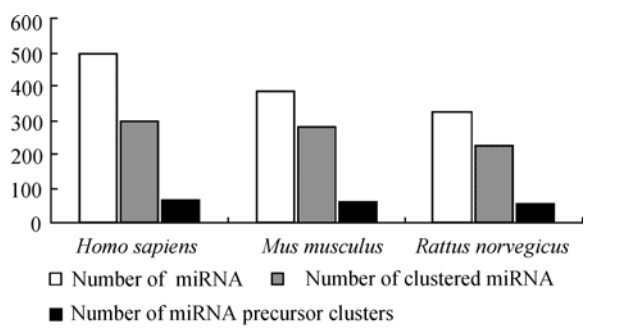

Figure 1 Total number of miRNA clusters in three mammalian genomes. (Source: http://www.mirz.unibas.ch/smiRNAdb/ PreClust.html)

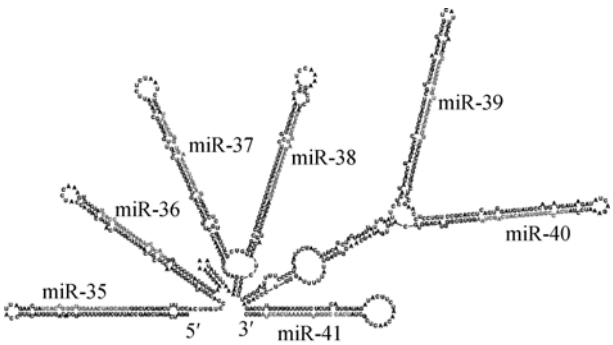

Figure 2 Secondary structure of the miR-35-41 cluster in *C. elegans*[28].

2 Expression and function of miRNA clusters

Since the two heterochronic regulatory miRNAs-*lin-4* and *let-7* were discovered in *C.elegans*[12,29], numerous studies have suggested the important roles of miRNAs in nearly all aspects of biological functions. Although miRNA targets could be predicted using bioinformatic approaches[30], only a few miRNAs have been studied in detail. For instance, a pancreatic islet-specific miR-375 modulates glucose-mediated insulin secretion by regulating the expression of myotrophin (Mtpn), which stimulates protein synthesis in myocytes[31]. A cardiac-specific miR-208 controls cardiac growth and Thyroid

hormone receptor associated protein 1 (THRAP1) expression in response to stress and hormonal signaling[16].

At present, most studies focus on the function of single miRNA, while studies on the expression and function of miRNA clusters are rare. There has been Accumulating evidence suggesting that clustered miRNA genes are often, though not always, located in a polycistron[4,14,21,32], coexpressed with neighboring miRNAs and host genes[33]. However, there were exceptions. In a study conducted by Lu et al.[34], through analysing the expression level of a miR-310/311/312/313 cluster, they found the expression level of miR-313 was notably different than that of three other members. Yu et al.[35]demonstrated that for the expression profile of 51 identified human miRNA clusters, 39 miRNA clusters showed the consistent expression of miRNAs in a single cluster, while the remaining miRNA clusters indicated the differential expression of members in a single cluster. Partially paralogous miRNA clusters on different chromosomes displayed identical expressions. The expression consistency of most miRNA clusters implies that the paralogous miRNA clusters may have common *cis*-regulatory elements, resulting in a cooperated function for those clusters. For other miRNA clusters with inconsistent expressions, they may be subject to different transcriptional or maturation processes. For example, Xu et al.[36] found that there were different transcriptional elements in the miR-212 cluster, the miR-363 cluster and the miR-382 cluster in mice.

Provided that miRNA clusters facilitate their coregulation, what benefits does the biological system receive from this arrangement? It seems that the clustered miRNAs are essential in regulating a complex cell signaling network, which is more efficient and complicated than the regulatory pattern mediated by discrete miRNAs.

In the study of Liu et al.[37], the miR-16 family effectively arrests cell division processes through regulating a series of cell cycle related proteins, including cyclin D1/D2/D3, E1/E2, and CDK2/4/6. With the combination of experiments and computational prediction, Xu et al.[36] suggested that by controlling three signaling factors-insulin receptor substrate 1 (Irs1), Ras p21 protein activator 1 (Rasa1) and growth factor receptor bound protein 2 (Grb2), the miR-183-96-182 cluster monitored the entire insulin signaling pathway in mice. Compared with the single miR-375- mediated regulation of insulin secretion[31], clustered miRNAs-mediated modulation is more likely to be efficient. Other studies on Drosophila embryonic development also revealed that the miR-12/miR-283/miR-30 clusters controlled the hedgehog signaling pathway[38], the miR-310s family regulated patterning in the morphogenesis and the miR-2/6/11/13/308 family was required for repressing embryonic apoptosis, as well as ensuring normal embryonic development[39]. Therefore, although the exact mechanisms underlying the clustering of miRNAs are largely unknown, it is suggested that the cluster arrangement of miRNAs is more efficient for regulating the complex gene network. The clustering miRNA homologs (miRNA family) are likely to constitute redundant self-protection[35], because in case one (or more) member of the homologs did not survive under selective pressure, other members at least would continuously act in order to maintain homeostasis.

3 Evolution of miRNA clusters

Most miRNAs are highly conserved between species because of functional constraints, reflected by not only the conservation of mature miRNA sequences, but also the flanking sequences necessary for the formation of secondary structures[40], which allows the homology searching for miRNAs across species. The classic example is the study of Berezikov et al.[41], which took advantage of the phylogenetic shadowing approach to identify human novel miRNA genes in comparison with other non-human primates.

For miRNA clusters, in addition to sequence homology, other evolutionary features including synteny, duplication on chromosomes and structural variations have been under scrutiny. The following sections will explore the evolutionary patterns of miRNA clusters.

In general there are four fundamental mechanisms underlying the origin and evolution of new genes—gene duplication, transposition and retrotransposition, exon shuffling and gene lateral transfer[42]. For miRNA genes, how do the four mechanisms drive the evolution of miRNA clusters? Zhang et al.[43] discovered tandem duplications that accelerated the evolution of an X-linked miR-506 cluster in primates[44]. Yu et al.[35] found that based on the homologous analysis of 38 miRNA clusters, partial duplications from an ancestral gene had driven the formation of the miRNA clusters. Further homologous analysis between 38 miRNA clusters demonstrated that the paralogs were present for 26 miRNA clusters,

where three miRNA gene families were detected—the first family composed of an let-7a-1 cluster，an let-7a-3 cluster and an miR-98 cluster, the second family made up of an miR-29c cluster and an miR-29a cluster, an miR-15a cluster and an miR-6-1 cluster comprising the third family. For each family, more than 90% of the sequence identities and synteny on chromosomes were retained between members in a single cluster and between clusters wihin a family. The results suggest that miRNA clusters (or miRNA families) are generated through duplication. In the studies of molecular evolution of miR-17 clusters[45] and phylogenetic evaluation of miRNA clusters in metazoan genomes[46], it was proposed that tandem duplication and segmental duplication were the key mechanisms for promoting the evolution of miRNA clusters. Studies of plants also revealed the two duplication processes could drive the evolution of miRNA clusters or families[47,48]. Following is an illustration of the duplication mechanism by which miRNA clusters are created (Figure 3).

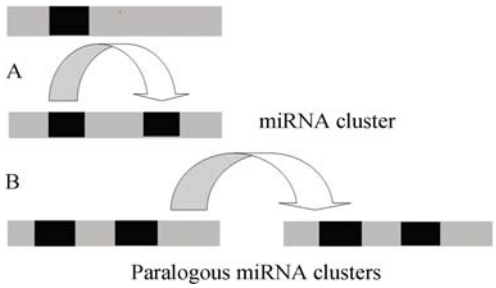

Figure 3 Evolutionary processes of miRNA clusters. A, tandem duplication; B, segmental duplication. Black boxes, miRNA genes.

In addition to duplication events, other types of evolutionary processes may also drive the evolution of miRNA clusters. Piriyapongsa et al.[49] found that 55 miRNAs out of the analyzed 462 human miRNAs were identical with transposition elements, indicating that those miRNAs were derived from transposition events. Zhang et al.[50] uncovered a primate-specific miRNA family, whose rearrangements on chromosome 19 were caused by *Alu* elements, resulting in the species-specific patterns of miRNA gain and loss. Similar results of the birth and death of miRNA genes were also indicated in Drosophila[34,51]. Consequently, transposition or retrotransposition-mediated evolution of miRNA clusters is a common mechanism.

Nuclear-containing mitochondrial peudogenes are acquired through a horizontal DNA transfer event in eukaryotes. When it comes to miRNA genes, it is not known whether it is possible to generate miRNA clusters through horizontal DNA transfer event. In the study of examining the potential relationship between nuclear mitochondrial DNA (NUMTs) and noncoding DNAs, the results showed no significant correlation between them[52], implying it was impossible to generate miRNA clusters through horizontal gene transfer, because the processes of integrating invaded sequences into the host genome which was performing regulatory roles in the host were extremely restricted by natural selection[53].

In conclusion, although the functional consequences of the clustering of miRNA genes are largely unknown, studies of the expression and function of miRNA clusters, as well as of evolutionary patterns, have suggested that the clustering of miRNA genes is the result of the gradual accumulation and fixation which occurs during natural selection, rather than duo to random events.

4 Case study

To further unravel the adaptive gain-and-loss alternation of clustering miRNAs during evolution we describe several case studies illustrating the origin of lineage-specific miRNAs.

Darwin positive selection is the primary process which causes rapid evolution of genes and the generation of novel genes. The origin and evolution of miRNA genes are substantially concerned with the discovery of many miRNAs. It is hypothesized that coordinated relation of miRNAs with their targets is the main cause for *de novo* generation of miRNA genes[47,54]. Lu et al.[34] found that in 12 species of the Drosophila genus, at least 5 miRNA genes were newly emerged, of which 4 miRNAs (miR-310/311/312/313) form a cluster. Furthermore, by estimating the divergent time, they confirmed that this miRNA cluster had originated during the branching of *Subgenus drosophila* and *Subgenus sophophora*. The fifth one (miR-303) appeared to be a very young gene, originating *de novo* from a non-miRNA sequence during the generation of a Drosophila subgroup. It has been reported that the miR-310 cluster played a critical role in the morphogenesis of later embryonic development[39]. Therefore, it suggests that the miR-310 cluster in the Drosophila subgenus would be a potential cause for the divergence of species. The study of the large–scale sequencing of all small RNAs in the

Drosophila subgenus also suggested the frequent *de novo* generation of miRNA genes. The birth and death of miRNA genes are common phenomena during divergence and speciation. Especially, during the speciation of *Drosophila simulans*, about 12 miRNA genes per Myr (million years) were created, and the net generation of miRNA genes is around 0.3 under a selective sweep[51]. Taken together, besides the key roles of protein–coding genes in the characterization of phenotypes, miRNA genes also play critical roles in regulatory genomes.

The studies of primates also indicated the rapid evolution of miRNA clusters with adaptive species-specific amplification or deletion[43,50], further demonstrating the genomically regulatory functions of miRNA clusters during primate evolution.

5 Perspectives

The coordination of miRNA genes is likely to be a driving force for the evolution of miRNA clusters. As part of the *dark mass* in the genome, it is crucial to reveal the origin and dynamics of miRNA clusters with coordinated regulatory functions. Therefore, it is necessary to utilize the phylogenomic approach to study the miRNA cluster regulatory network, i.e. to dissect whether the birth and death of any specific miRNA cluster or cluster member may lead to the altered target gene repertoire and the consequences of the changed miRNA-target interaction. Furthermore, dissecting the pattern of the miRNA regulatory network will also benefit drug discovery as well as elucidating mechanisms underlying the onset of complex diseases.

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