

Biology

Query paper:

Title: MicroRNAs: genomics, biogenesis, mechanism, and function

Abstract: MicroRNAs (miRNAs) are endogenous ~22 nt RNAs that can play important regulatory roles in animals and plants by targeting mRNAs for cleavage or translational repression. Although they escaped notice until relatively recently, miRNAs comprise one of the more abundant classes of gene regulatory molecules in multicellular organisms and likely influence the output of many protein-coding genes.

Candidate papers:

1. **Title:** The small RNA profile during *Drosophila melanogaster* development

Abstract: Small RNAs ranging in size between 20 and 30 nucleotides are involved in different types of regulation of gene expression including mRNA degradation, translational repression, and chromatin modification. Here we describe the small RNA profile of *Drosophila melanogaster* as a function of development. We have cloned and sequenced over 4000 small RNAs, 560 of which have the characteristics of RNase III cleavage products. A nonredundant set of 62 miRNAs was identified. We also isolated 178 repeat-associated small interfering RNAs (rasiRNAs), which are cognate to transposable elements, satellite and microsatellite DNA, and Suppressor of Stellate repeats, suggesting that small RNAs participate in defining chromatin structure. rasiRNAs are most abundant in testes and early embryos, where regulation of transposon activity is critical and dramatic changes in heterochromatin structure occur.

2. **Title:** Regulation of Flowering Time and Floral Organ Identity by a MicroRNA and Its APETALA2-Like Target Genes

Abstract: MicroRNAs (miRNAs) are ~21-nucleotide noncoding RNAs that have been identified in both animals and plants. Although in animals there is direct evidence implicating particular miRNAs in the control of developmental timing, to date it is not known whether plant miRNAs also play a role in regulating temporal transitions. Through an activation-tagging approach, we demonstrate that miRNA 172 (miR172) causes early flowering and disrupts the specification of floral organ identity when overexpressed in *Arabidopsis*. miR172 normally is expressed in a temporal manner, consistent with its proposed role in flowering time control. The regulatory target of miR172 is a subfamily of APETALA2 (AP2) transcription factor genes. We present evidence that miR172 downregulates these target genes by a translational mechanism rather than by RNA cleavage. Gain-of-function and loss-of-function analyses indicate that two of the AP2-like target genes normally act as floral repressors, supporting the notion that miR172 regulates flowering time by downregulating AP2-like target genes.

3. **Title:** Fragile X-related protein and VIG associate with the RNA interference machinery

Abstract: RNA interference (RNAi) is a flexible gene silencing mechanism that responds to double-stranded RNA by suppressing homologous genes. Here, we report the characterization of RNAi effector complexes (RISCs) that contain small interfering RNAs and microRNAs (miRNAs). We identify two putative RNA-binding proteins, the *Drosophila* homolog of the fragile X mental retardation protein (FMRP), dFXR, and VIG (Vasa intronic gene), through their association with RISC. FMRP, the product of the human fragile X locus, regulates the expression of numerous

mRNAs via an unknown mechanism. The possibility that dFXR, and potentially FMRP, use, at least in part, an RNAi-related mechanism for target recognition suggests a potentially important link between RNAi and human disease.

4. **Title:** Killing the messenger: short RNAs that silence gene expression

Abstract: Short interfering RNAs can be used to silence gene expression in a sequence-specific manner in a process that is known as RNA interference. The application of RNA interference in mammals has the potential to allow the systematic analysis of gene expression and holds the possibility of therapeutic gene silencing. Much of the promise of RNA interference will depend on the recent advances in short-RNA-based silencing technologies.

5. **Title:** Gene silencing in worms and fungi

Abstract: The introduction into cells of foreign nucleic acid molecules can induce sequence-specific gene silencing in some organisms. Here we show that two distantly related organisms, the nematode *Caenorhabditis elegans* and the fungus *Neurospora crassa*, which have quite different mechanisms of gene silencing, both use a similar protein to control the process. This suggests that they may share an ancestral mechanism that evolved to protect the genome against invasion by foreign DNA.

6. **Title:** siRNAs can function as miRNAs

Abstract: With the discovery of RNA interference (RNAi) and related phenomena, new regulatory roles attributed to RNA continue to emerge. Here we show, in mammalian tissue culture, that a short interfering RNA (siRNA) can repress expression of a target mRNA with partially complementary binding sites in its 3' UTR, much like the demonstrated function of endogenously encoded microRNAs (miRNAs). The mechanism for this repression is cooperative, distinct from the catalytic mechanism of mRNA cleavage by siRNAs. The use of siRNAs to study translational repression holds promise for dissecting the sequence and structural determinants and general mechanism of gene repression by miRNAs.

Exemplary analysis:

1. **Relevance:** It supports the query paper's discussion on the abundance and variety of miRNAs across multicellular organisms and their potential regulatory roles at different developmental stages.

Reason for Citation: This paper is likely cited because it provides foundational evidence of the diversity and developmental regulation of small RNAs, including miRNAs, in a model organism, *Drosophila melanogaster*.

2. **Relevance:** This study is relevant because it demonstrates a specific functional role of a miRNA (miR172) in plant development, particularly in flowering time and floral organ identity.

Reason for Citation: It exemplifies how miRNAs can influence the output of protein-coding genes, a central theme of the query paper, by showing miR172's effect on AP2-like target genes.

3. **Relevance:** The association of proteins related to the RNA interference machinery with miRNAs underscores the complexity of gene regulation by small RNAs and supports the query paper's discussion on the mechanism of miRNA action.

Reason for Citation: The inclusion of this paper likely serves to highlight the intersection of

miRNA pathways with other RNA-based regulatory mechanisms and their potential implications for human disease.

4. **Relevance:** This paper provides a broad overview of RNA interference (RNAi) and its potential for gene silencing, which is relevant to understanding the functional mechanisms of miRNAs.

Reason for Citation: Since miRNAs can guide RNA-induced silencing complexes to target mRNAs for repression or cleavage, citing this work helps frame the broader context of RNA-based gene regulation discussed in the query paper.

5. **Relevance:** It supports the idea that miRNAs and their mechanisms of action are part of a broader, ancient system of gene regulation that spans from fungi to animals, reinforcing the significance of studying miRNAs within a wide biological context.

Reason for Citation: By citing this paper, the query paper acknowledges the evolutionary conservation of RNA-based gene silencing mechanisms across different organisms.

6. **Relevance:** It supports the query paper's exploration of miRNA mechanisms by showing that siRNAs can also mediate translational repression through partial complementarity to target mRNAs, similar to miRNAs. This citation underscores the versatility and complexity of small RNA-mediated gene regulation.

Reason for Citation: This study is cited because it bridges the functional similarities between siRNAs and miRNAs, two classes of small RNAs involved in gene silencing.

Exemplary ranking: Ranked order: paper 1, paper 2, paper 4, paper 6, paper 3, paper 5

1. **Explanation:** As it provides evidence of the diversity and developmental regulation of miRNAs in a model organism, this paper is important for studies focusing on the biogenesis and functional roles of miRNAs during development.
2. **Explanation:** This study is specific to plant miRNAs but is crucial for understanding miRNA function in plant development. It is particularly relevant for research exploring miRNA roles in developmental processes and gene expression in plants.
3. **Explanation:** This paper provides a broad overview of RNA interference and gene silencing mechanisms, which are central to understanding miRNA function. It is foundational for anyone studying miRNA mechanisms and their implications in gene regulation.
4. **Explanation:** Given the functional similarities between siRNAs and miRNAs, this paper is crucial for understanding the versatility and complexity of RNA-mediated gene regulation. It directly supports the exploration of miRNA mechanisms, making it highly relevant for follow-up research.
5. **Explanation:** This paper highlights the intersection of miRNA pathways with other RNA-based regulatory mechanisms, which is essential for comprehending the broader context of miRNA function in gene regulation and disease.
6. **Explanation:** While this paper supports the evolutionary conservation of RNA-based gene silencing, it might be considered more specialized for research focusing on the evolutionary aspects of miRNA function across different organisms.