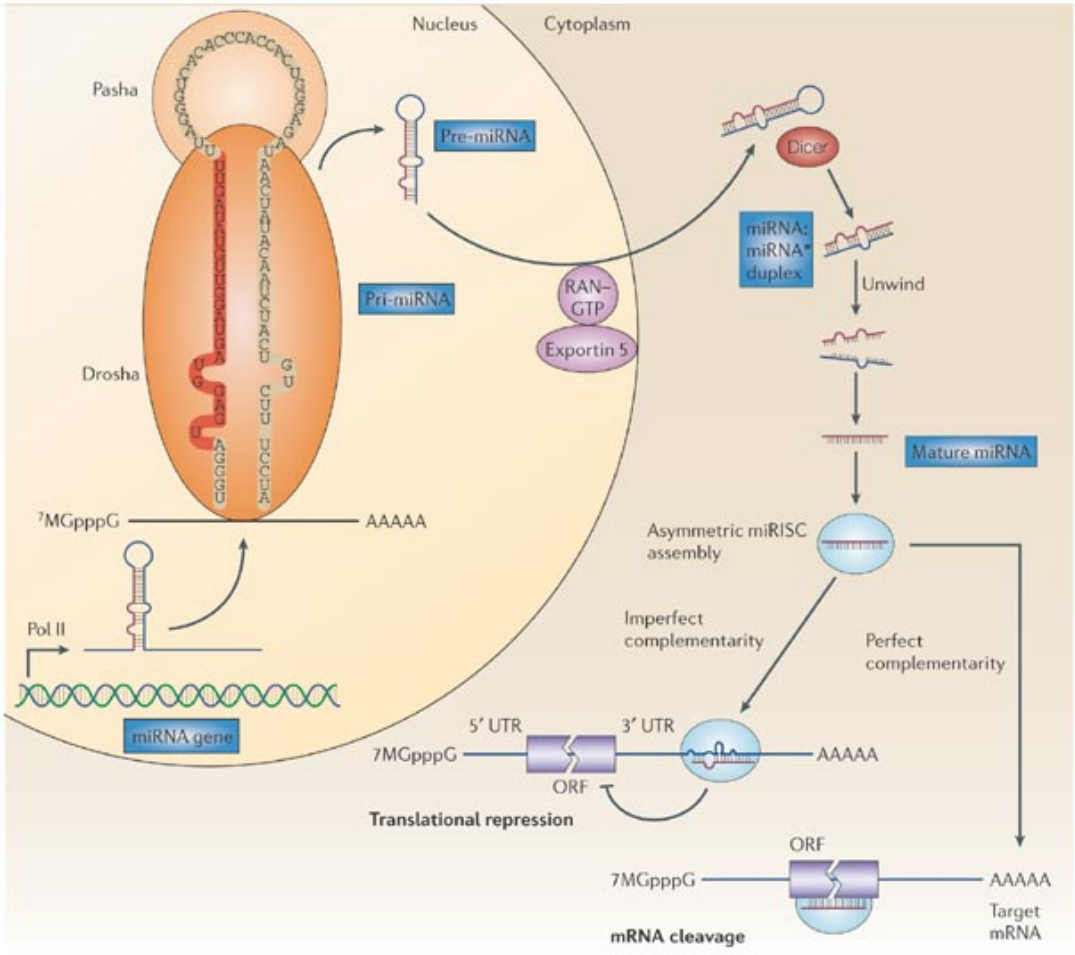
Micro RNAs

Criteria for micro RNA annotation

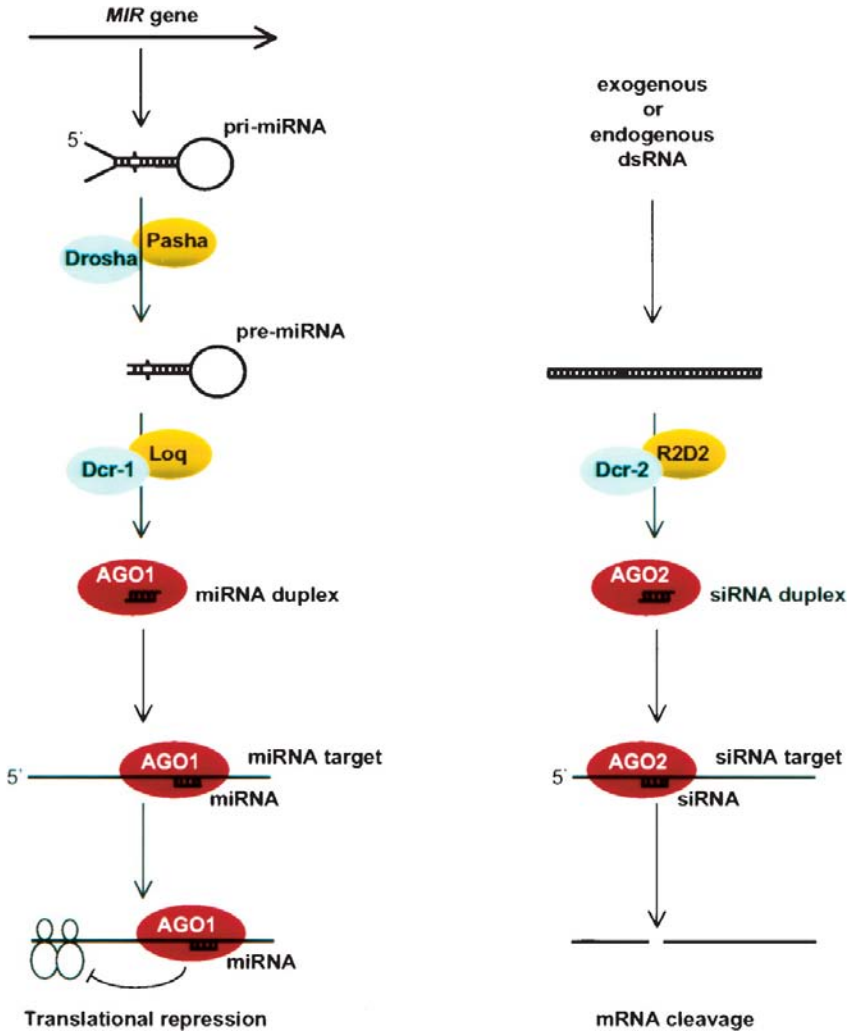
- detection of a ≅ 22 nt RNA transcript by Northern blotting or in a size-fractionated library
- prediction of a potential fold-back precursor which contains the 22 nt sequence in one arm of the hairpin, 60 – 80 in animals, in plants may be up to a few hundereds.
- phylogenetic conservation of the ≅ 22 nt and the precursor structure
- detection of increased precursor accumulation in organism with reduced Dicer function



Esquela-Kerscher *et al. Nature Reviews Cancer* **6**, 259–269 (April 2006) | doi:10.1038/nrc1840

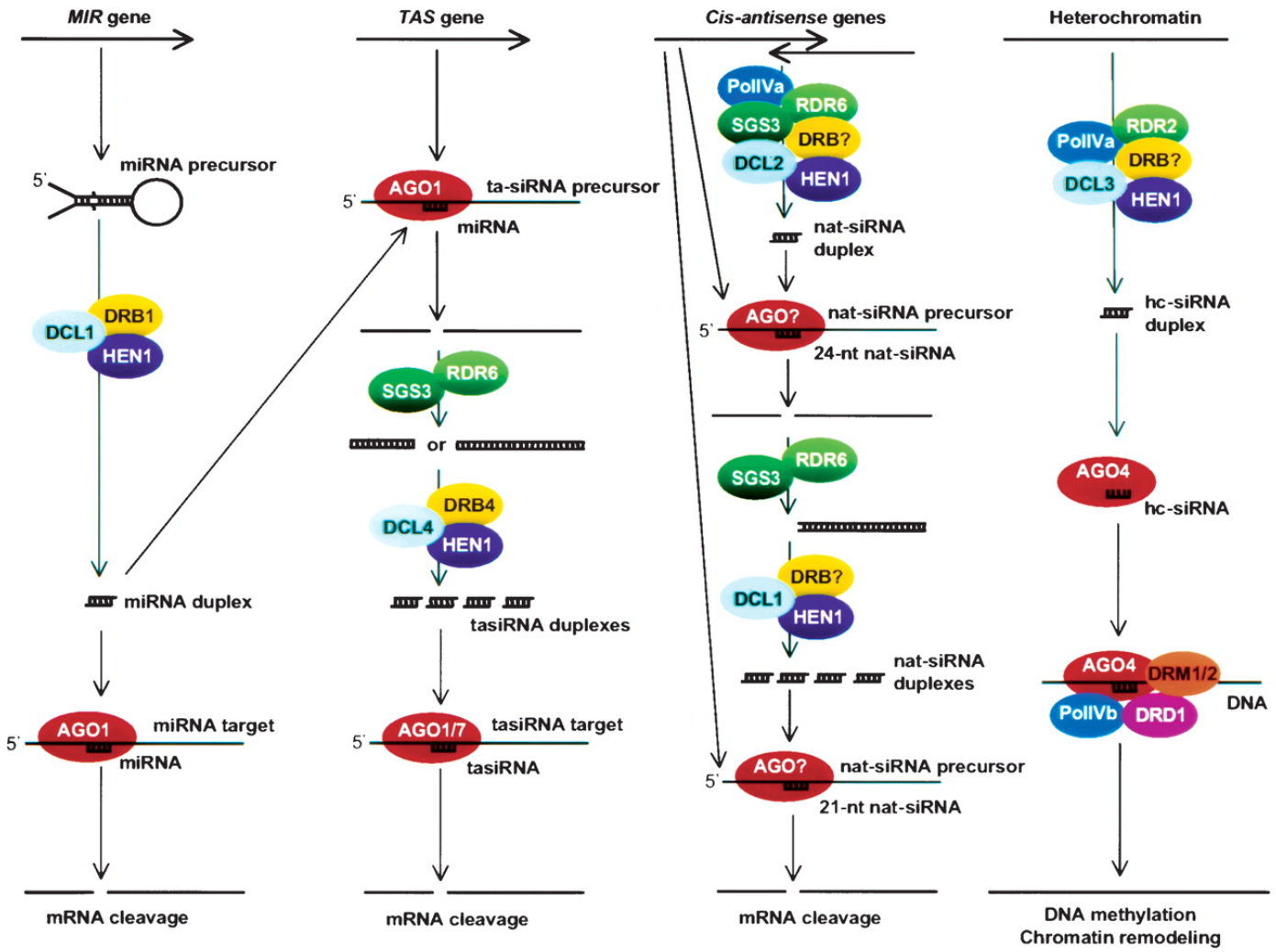
nature REVIEWS CANCER

The biogenesis of microRNAs. MicroRNA (miRNA) genes are generally transcribed by RNA Polymerase II (Pol II) in the nucleus to form large pri-miRNA transcripts, which are capped (7MGpppG) and polyadenylated (AAAAA). These pri-miRNA transcripts are processed by the RNase III enzyme Drosha and its co-factor, Pasha, to release the ~70-nucleotide pre-miRNA precursor product. (Note that the human let-7a-1 miRNA is shown here as an example of a pre-miRNA hairpin sequence. The mature miRNA sequence is shown in red.) RAN–GTP and exportin 5 transport the pre-miRNA into the cytoplasm. Subsequently, another RNase III enzyme, Dicer, processes the pre-miRNA to generate a transient ~22- nucleotide miRNA:miRNA* duplex. This duplex is then loaded into the miRNA-associated multiprotein RNA-induced silencing complex (miRISC) (light blue), which includes the Argonaute proteins, and the mature single-stranded miRNA (red) is preferentially retained in this complex. The mature miRNA then binds to complementary sites in the mRNA target to negatively regulate gene expression in one of two ways that depend on the degree of complementarity between the miRNA and its target. miRNAs that bind to mRNA targets with imperfect complementarity block target gene expression at the level of protein translation (lower left). However, recent evidence indicates that miRNAs might also affect mRNA stability (not shown). Complementary sites for miRNAs using this mechanism are generally found in the 3‘ untranslated regions (3’ UTRs) of the target mRNA genes. miRNAs that bind to their mRNA targets with perfect (or nearly perfect) complementarity induce target-mRNA cleavage (lower right). miRNAs using this mechanism bind to miRNA complementary sites that are generally found in the coding sequence or open reading frame (ORF) of the mRNA target.



miRNA and siRNA pathways in animals. The pathways in *Drosophila* are shown as exampled, owing to the remarkable dissection of the pathway in this organism. A color code is used to indicate members of the same gene family. Translation repression and mRNA cleavage steps involve additional components that are not represented in this figure.

Herve Vaucheret Genes Dev. 2006; 20: 759-771



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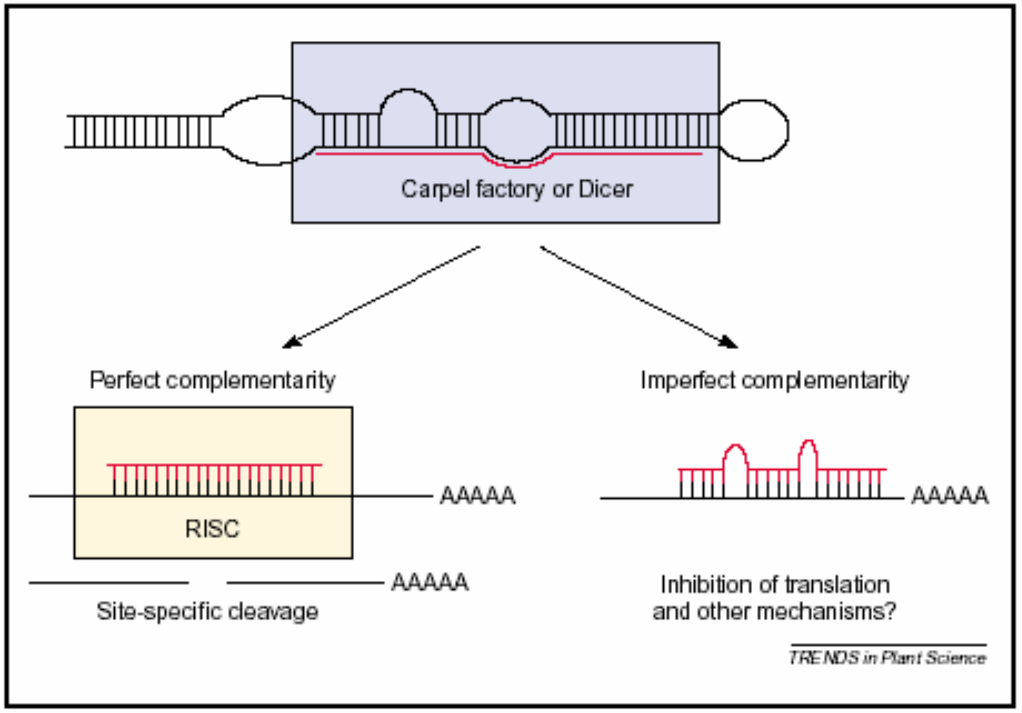
miRNA, ta-siRNA, nat-siRNA, and hc-siRNA pathways in plants. The same color code as in Figure 1 is used to indicate members of the same gene family. Question marks indicate that a member is likely to play a role in the pathway, but the identity of the protein has not been experimentally determined. HYL1 is referred to as DRB1 for clarity. The arrow between the miRNA and ta-siRNA pathways indicates that the miRNA pathway is required for the proper functioning of the ta-siRNA pathway. The multiple arrows emanating from the sense gene in the nat-siRNA pathway indicate that the RNA transcribed from this gene is used at various steps in this pathway. DNA modification steps in the hc-siRNA pathway involve additional locus-specific components that are not represented in this figure.

ta-siRNA: derive from *TAS* genes, transcribed in long primary transcript which do not code for a protein and which are first cut by a miRNA. This serves as template for reverse transcription into dsRNA, which then are processed by DCL-4 (dicer-like 4).

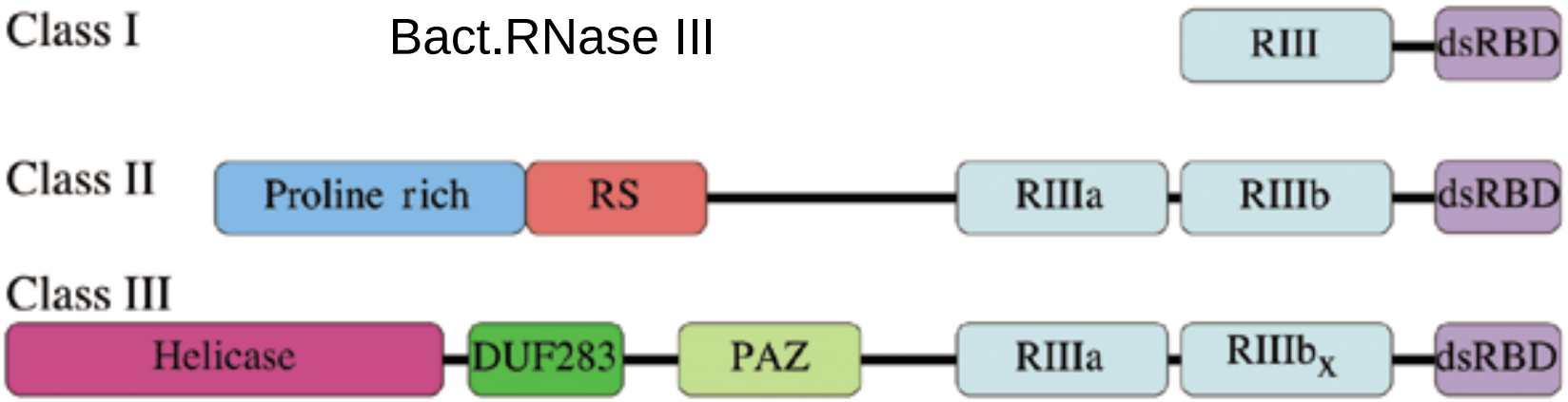
nat-siRNA: endogenous siRNAs, derive from natural cis-antisense transcripts, use DCL-2, are 24 nt long, approx. 2000 cis-antisense transcript in Arabidopsis.

Modes of action of miRNA in plants



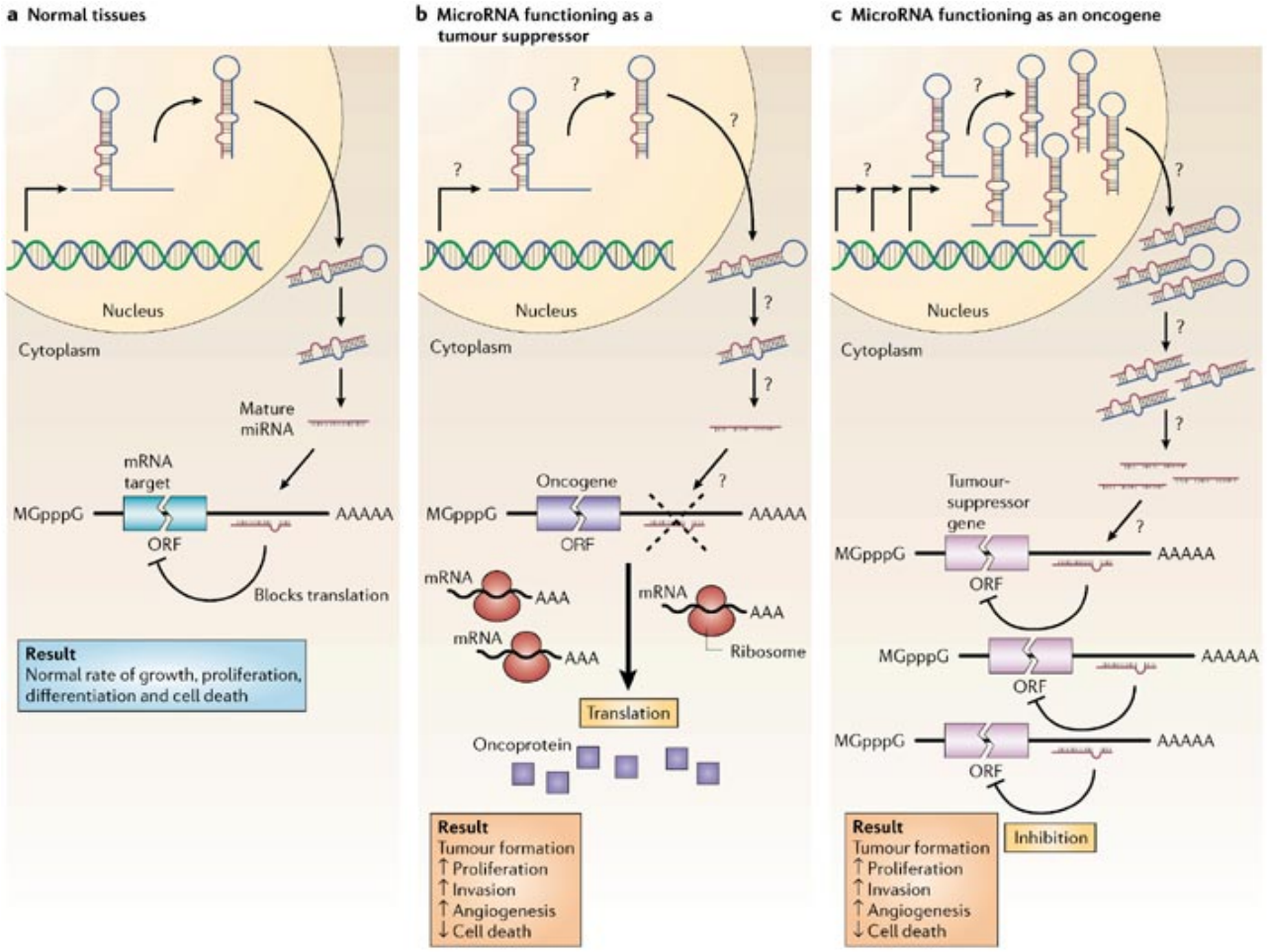
Jones, L., Trends in Plant Sciences, 7,473, 2002

ENZYME STRUCTURE – RNase III



Class II: Drosha
Class III dicer

MicroRNAs can function as tumour suppressors and oncogenes



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MicroRNAs can function as tumour suppressors and oncogenes. a | In normal tissues, proper microRNA (miRNA) transcription, processing and binding to complementary sequences on the target mRNA results in the repression of target-gene expression through a block in protein translation or altered mRNA stability (not shown). The overall result is normal rates of cellular growth, proliferation, differentiation and cell death.
b | The reduction or deletion of a miRNA that functions as a tumour suppressor leads to tumour formation. A reduction in or elimination of mature miRNA levels can occur because of defects at any stage of miRNA biogenesis (indicated by question marks) and ultimately leads to the inappropriate expression of the miRNA-target oncoprotein (purple squares). The overall outcome might involve increased proliferation, invasiveness or angiogenesis, decreased levels of apoptosis, or undifferentiated or de-differentiated tissue, ultimately leading to tumour formation.
c | The amplification or overexpression of a miRNA that has an oncogenic role would also result in tumour formation. In this situation, increased amounts of a miRNA, which might be produced at inappropriate times or in the wrong tissues, would eliminate the expression of a miRNA-target tumour-suppressor gene (pink) and lead to cancer progression. Increased levels of mature miRNA might occur because of amplification of the miRNA gene, a constitutively active promoter, increased efficiency in miRNA processing or increased stability of the miRNA (indicated by question marks). ORF, open reading frame.

Table 1 | **MicroRNAs that are associated with human cancers**

miRNA	Gene loci	Cancer association	Function	References
miR-15a, miR-16-1	Chromosome 13q14	Frequently deleted or downregulated in B-cell chronic lymphocytic leukemia; negatively regulates the anti-apoptotic gene *BCL2*	TS	93,94
miR-143, miR-145	Chromosome 5q32–33	Decreased abundance in colorectal cancer; downregulated in breast, prostate, cervical and lymphoid cancer cell lines; miR-145 is decreased in breast cancer	TS	84,97
miR-21	Chromosome 17q23.2	Anti-apoptotic factor; upregulated in glioblastomas and breast cancer	OG	84,100,101
let-7 family members	Multiple loci	Negatively regulate the Ras oncogenes; direct cell proliferation and differentiation; decreased abundance in lung cancer	TS	81,82
miR-142	Chromosome 17q22	A t(8;17) translocation that places the *MYC* oncogene downstream of the *mir-142* hairpin, resulting in an aggressive B-cell leukemia that is due to MYC overexpression	N/A	41
BIC/miR-155	Chromosome 21q21	Upregulated in paediatric Burkitt, Hodgkin, primary mediastinal and diffuse large-B-cell lymphomas; upregulated in human breast cancer	OG	84,105–108
miR-17–19b cluster	Chromosome 13q31–32	Upregulated by MYC; negatively modulates the *E2F1* oncogene; loss of heterozygosity of this cluster is found in hepatocellular carcinoma; overexpressed in B-cell lymphomas	TS/OG	109,110

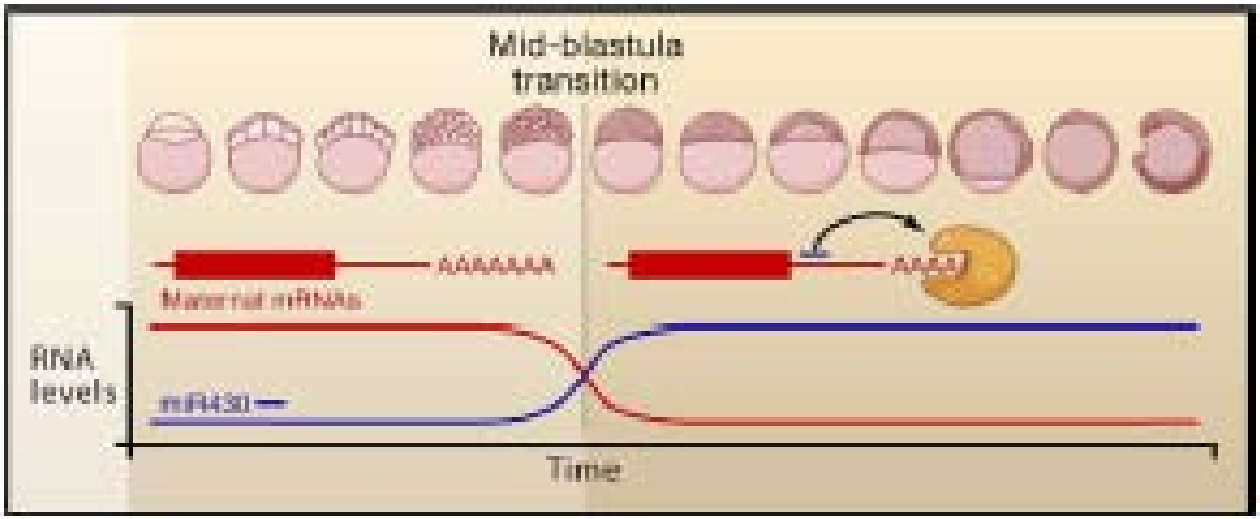
N/A, not applicable; OG, oncogene; TS, tumour suppressor.

At a glance

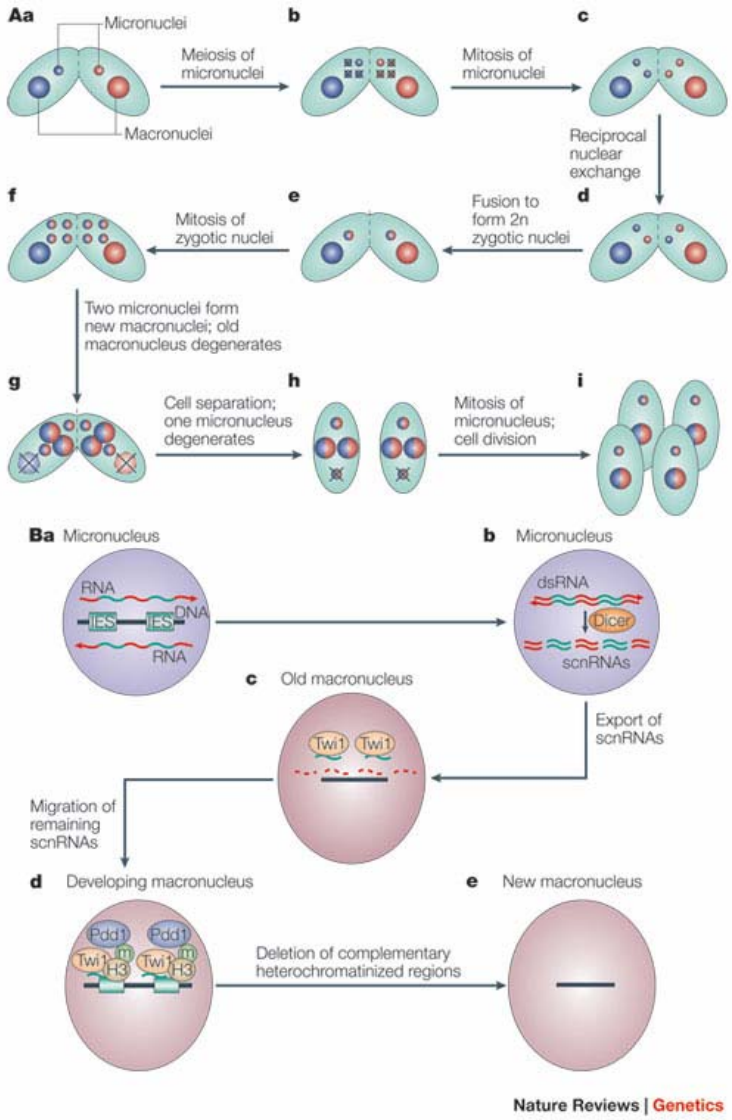
- MicroRNAs (miRNAs) are an abundant class of negative gene regulators that have been shown to control a wide range of biological functions such as cellular proliferation, differentiation and apoptosis.
- About half of the annotated human miRNAs map within fragile regions of chromosomes, which are areas of the genome that are associated with various human cancers.
- Recent evidence indicates that miRNAs can function as tumour suppressors and oncogenes, and they are therefore referred to as 'oncomirs'. Factors that are required for the biogenesis of miRNAs have also been associated with various cancers and might themselves function as tumour suppressors and oncogenes.
- Expression profiling of miRNAs has been shown to be a more accurate method of classifying cancer subtypes than using the expression profiles of protein-coding genes. The differential expression of certain miRNAs in various tumours might become a powerful tool to aid in the diagnosis and treatment of cancer.
- Gene therapies that use miRNAs might be an effective approach to blocking tumour progression. miRNAs such as let-7, which has been shown to negatively regulate the Ras oncogenes, and miR-15 and miR-16, which negatively regulate *BCL2*, are promising candidates for cancer treatment.

miR430 Promotes Decay of Maternal mRNAs during Zebrafish Development

After fertilization until the mid-blastual transition, miR430 abundance is low and maternal mRNA messages with a miR430 complementary motif in their 3‘ UTR are stable. Once zygotic transcription begins, miR430 expression is activated, and its levels rise. Binding of miR430 to the 3‘ UTR of maternal messages leads to removal of the poly(A) tail, thereby triggering a massive and synchronous degradation of these messages.



Weigel and Izaurralde, 2006, Cell *124*, 1118,



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Vegetative cells of T. thermophila contain one micronucleus and one macronucleus. Conjugation begins with pairing of complementary mating types (a). The micronucleus undergoes meiosis to produce four haploid nuclei, three of which degenerate (b). The remaining haploid micronucleus divides mitotically (c). This is followed by reciprocal nuclear exchange (d) and fusion to form a new diploid zygote nucleus (e). The zygote nucleus undergoes two mitotic divisions (f). From the four products of these divisions, two develop into new macronuclei as the old macronucleus degenerates (g). It is during this period (f, g) that internal eliminated segment (IES) elimination and other genome alterations occur. Cells then separate and one micronucleus degenerates (h). The remaining micronucleus divides mitotically and subsequent cell divisions produce four daughter vegetative cells (i). RNA-scan model for IES elimination. Micronuclear IES sequences destined for elimination during development of the new macronucleus are shown in green. Early in conjugation, the micronuclear genome is bidirectionally transcribed (a). The resulting dsRNA is processed by Dicer into small RNAs, termed scan RNAs (scnRNAs) (b). The scnRNAs are exported into the old macronucleus, and those that can base pair to genomic DNA (red dotted lines) are degraded. The remaining scnRNAs, which correspond to sequences eliminated in the previous conjugation, migrate to the developing new macronucleus (c) and induce H3K9 methylation of the homologous IES sequences (d), which are then deleted (e). Twi1 is a Piwi-related protein that associates with small RNAs in both the old and new macronuclei. Programmed DNA degradation protein 1 (Pdd1) binds through its chromodomain to H3 methylated on lysine 9 and is essential for DNA elimination.