MicroRNA (miRNA) biogenesis

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Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Reactome database release: 77

This document contains 1 pathway and 8 reactions (see Table of Contents)

1. **Transcription.** miRNA transcripts may come from autonomously transcribed genes, they may be contained in cotranscripts with other genes, or they may be located in introns of host genes. Most miRNAs are transcribed by RNA polymerase II, however a few miRNAs originate as RNA polymerase III cotranscripts with neighboring repetitive elements. The initial transcript, termed a primary microRNA (pri-miRNA), contains an imperfectly double-stranded region within a hairpin loop. Longer sequences extend from the 5' and 3' ends of the hairpin and may also contain double-stranded regions.

2. **Cleavage by DROSHA.** The 5' and 3' ends of the pri-miRNA are removed during endoribonucleolytic cleavage by the DROSHA nuclease in a complex with the RNA-binding protein DGCR8 (the Microprocessor complex). The cleavage product is a short hairpin of about 60 to 70 nt called the pre-microRNA (pre-miRNA).

3. **Nuclear export by Exportin-5.** The resulting pre-miRNA is bound by Exportin-5 in a complex with Ran and GTP. The complex translocates the pre-miRNA through the nuclear pore into the cytoplasm.

4. **Cleavage by DICER1.** Once in the cytoplasm the pre-miRNA is bound by the RISC loading complex which contains DICER1, an Argonaute protein and either TARBP2 or PRKRA. DICER1 cleaves the pre-miRNA to yield an imperfectly double-stranded miRNA of about 21 to 23 nucleotides. At this stage the double-stranded miRNA has protruding single-stranded 3' ends of 2-3 nt.

5. **Incorporation into RNA-Induced Silencing Complex (RISC) and strand selection.** The double-stranded miRNA is passed to a Argonaute protein contained in the RISC loading complex. One strand, the passenger strand, will be removed and degraded; the other strand, the guide strand, will be retained and will guide the Argonaute:miRNA complex (RISC) to target mRNAs.
The human genome encodes 4 Argonaute proteins (AGO1 (EIF2C1), AGO2 (EIF2C2), AGO3 (EIF2C3), AGO4 (EIF2C4)), however only AGO2 (EIF2C2) can cleave target mRNAs with perfect or nearly perfect complementarity to the guide miRNA. For complexes that contain AGO2, cleavage of the passenger strand of the double-stranded miRNA accompanies removal of the passenger strand. Complexes containing other Argonautes may use a helicase to remove the passenger strand but this is not fully known. The resulting miRNA-loaded AGO2 is predominantly located in complexes with TARBP2 or PRKRA at the cytosolic face of the rough endoplasmic reticulum. AGO2, TARBP2, and DICER1 are also observed in the nucleus.

**Literature references**


**Editions**

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Pol II mediated transcription of microRNA genes

**Location:** MicroRNA (miRNA) biogenesis

**Stable identifier:** R-HSA-203901

**Type:** omitted

**Compartments:** nucleoplasm

Transcription of miRNA genes. Most miRNAs are transcribed by RNA polymerase II. The miRNAs may be autonomous transcription units or they may be located in other transcripts, including locations within introns and other untranslated regions. Of the polymerase II transcribed miRNAs, about 60% are located in introns of protein coding genes, 12% are in introns of non-coding RNAs, 18% are in exons of non-coding RNAs, and 10% uncertain.

A second class of miRNA genes are associated with Alu and other repetitive elements and are cotranscribed with these elements by RNA polymerase III. There are currently only a few proven examples of polymerase III transcribed miRNAs.

**Followed by:** Microprocessor complex cleaves pri-miRNA to pre-miRNA

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https://reactome.org
**Microprocessor complex cleaves pri-miRNA to pre-miRNA**

**Location:** MicroRNA (miRNA) biogenesis

**Stable identifier:** R-HSA-203893

**Type:** transition

**Compartments:** nucleoplasm

Nuclear processing by Drosha Microprocessor complex. The primary-microRNA (pri-miRNA) is recognized by the Microprocessor complex (Drosha:DGCR8) and both strands of the pri-miRNA are cleaved by Drosha near the free 5' and 3' ends of the pri-miRNA, that is, at the ends distal from the internal loop. The product is a double-stranded RNA having 2 nucleotides protruding at the 3' end and having an internal loop.

**Preceded by:** Pol II mediated transcription of microRNA genes

**Followed by:** Exportin-5 recognizes 3' overhang of pre-miRNA

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Exportin-5 recognizes 3' overhang of pre-miRNA

**Location:** MicroRNA (miRNA) biogenesis

**Stable identifier:** R-HSA-203922

**Type:** transition

**Compartments:** nucleoplasm

Exportin-5 binds pre-microRNAs having 2-nucleotide overhangs at the 3' end. Binding is independent of sequence and depends on GTP.

**Preceded by:** Microprocessor complex cleaves pri-miRNA to pre-miRNA

**Followed by:** Exportin complex translocates pre-miRNA to cytosol

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**Exportin complex translocates pre-miRNA to cytosol**

**Location:** MicroRNA (miRNA) biogenesis

**Stable identifier:** R-HSA-203906

**Type:** transition

**Compartments:** cytosol, nucleoplasm

Nuclear Export by Exportin-5. The pre-microRNA is bound by the Exportin-5:RanGTP complex in the nucleus and the complex is translocated through the nuclear pore into the cytoplasm. In the process GTP is hydrolyzed to GDP.

**Preceded by:** Exportin-5 recognizes 3' overhang of pre-miRNA

**Followed by:** BCDIN3D dimethylates 5' phosphate of pre-miR-145, Dicer cleaves pre-miRNA to yield duplex miRNA

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Dicer cleaves pre-miRNA to yield duplex miRNA

Location: MicroRNA (miRNA) biogenesis

Stable identifier: R-HSA-203862

Type: transition

Compartments: cytosol

Pre-miRNA binds the RISC loading complex (RLC), a complex containing DICER1, AGO2, and TARBP2 (TRBP). Alternative loading complexes contain AGO1, AGO3, or AGO4 rather than AGO2 and PRKRA (PACT) rather than TARBP2. The pre-miRNA substrate has an internal loop and a protruding 3' end created by cleavage by DROSHA:DGCR8. The DICER1:TRBP2 subcomplex or DICER1:PRKRA subcomplex recognizes this structure and the DICER1 component cleaves the pre-miRNA near the loop. The product is a double-stranded RNA of 21-25 nucleotides having 2-nucleotide protrusions at each 3' end. The products have 5' phosphates and 3' hydroxyl groups. Diffusion activity of TARBP2 and PRKRA along duplex RNA may enhance processing by DICER1.

Preceded by: Exportin complex translocates pre-miRNA to cytosol

Followed by: Duplex miRNA is loaded into Argonaute

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The duplex miRNA (designated miRNA-miRNA*) is reoriented on DICER1 after cleavage and then transferred from DICER1 to an Argonaute protein (AGO2 or, by inference, AGO1, AGO3, or AGO4) within the RISC loading complex. Particular Argonaute proteins do not appear to have significantly different populations of miRNAs, however Argonaute identity can affect the resulting length of the miRNA.

**Preceded by:** Dicer cleaves pre-miRNA to yield duplex miRNA

**Followed by:** Removal of miRNA passenger strand

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Removal of miRNA passenger strand

**Location:** MicroRNA (miRNA) biogenesis

**Stable identifier:** R-HSA-210805

**Type:** omitted

**Compartments:** cytosol

A short double-stranded RNA is passed from DICER1 to an Argonaute protein and rendered single-stranded by removal and loss of the passenger strand. All Argonautes (AGO1 (EIF2C1), AGO2 (EIF2C2), AGO3 (EIF2C3), AGO4 (EIF2C4)) can remove the passenger strand without cleaving it and most miRNAs are processed in this way. AGO2 (EIF2C2) can cleave the passenger strand of a subset of miRNAs that have no mismatches in the central region (Shin et al. 2008).

RNA helicase A associated with the RISC loading complex can facilitate removal of the passenger strand.

The mechanism that selects which strand is retained as the guide RNA is not well understood in humans. Overhanging nucleotides and strength of base-pairing at each end of the input duplex are observed to influence strand selection.

In cultured cells Argonaute proteins loaded with miRNAs or siRNAs are predominantly located in association with TARBP2 or PRKRA at the cytosolic face of the rough endoplasmic reticulum. In adult non-dividing cells most Argonaute-bound miRNAs are located in low molecular weight complexes but shift to larger complexes containing GW182 in response to phosphoinositide-3-kinase/mTOR signaling.

**Preceded by:** Duplex miRNA is loaded into Argonaute

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https://reactome.org
**BCDIN3D dimethylates 5' phosphate of pre-miR-145**

**Location:** MicroRNA (miRNA) biogenesis

**Stable identifier:** R-HSA-5578717

**Type:** transition

**Compartments:** cytosol

BCDIN3D transfers a methyl group from S-adenosylcysteine to the each of the 2 hydroxyl groups of the 5' phosphate of pre-miR-145 and pre-miR-23b (Xhemalce et al. 2012). The methylation eliminates the negative charges on the phosphate and thereby interferes with the recognition of pre-miRNAs by Dicer, inhibiting production of mature miR-145.

**Preceded by:** Exportin complex translocates pre-miRNA to cytosol

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