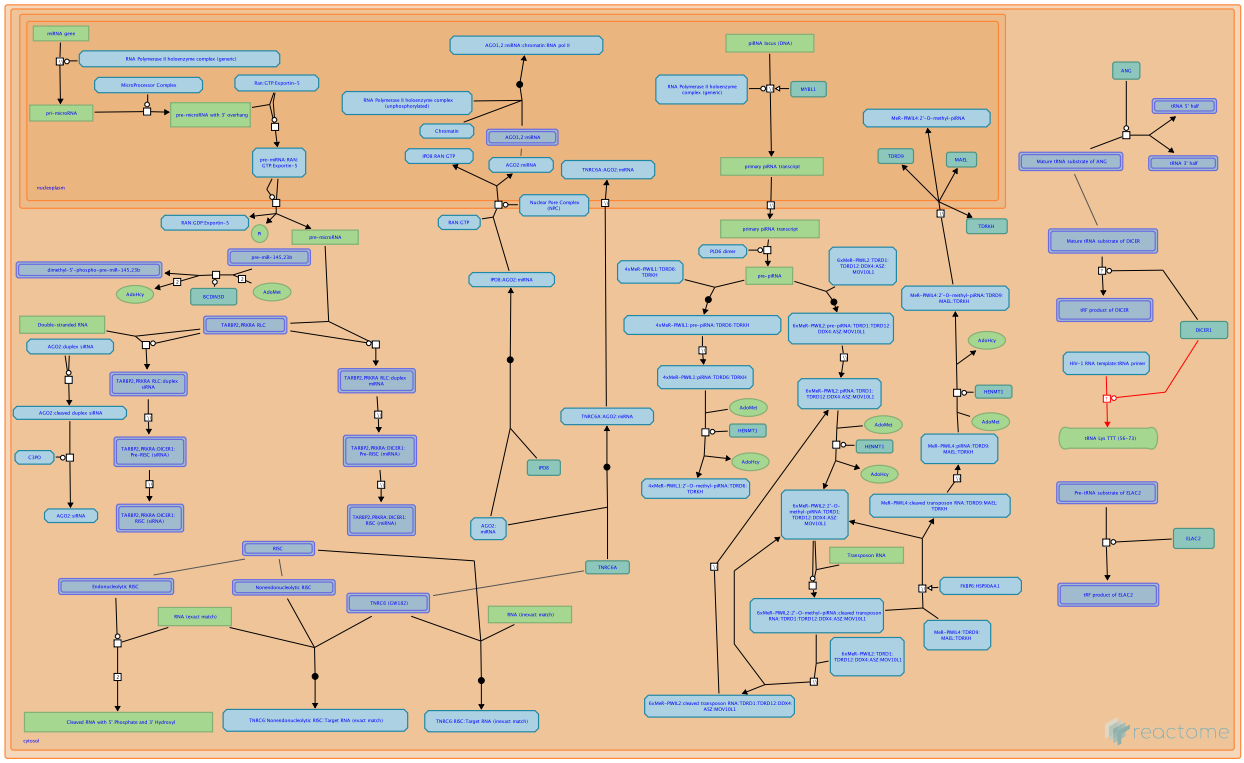
Gene Silencing by RNA



Basso, K., Dutta, A., Gopinathrao, G., Hannon, GJ., Karginov, F., Li, LC., May, B., Saito, K., Stroynowska-Czerwinska, A., Su, Z., Tomari, Y., Wilson, B.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

14/09/2021

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

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Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics, 33*, 3461-3467. ↗

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

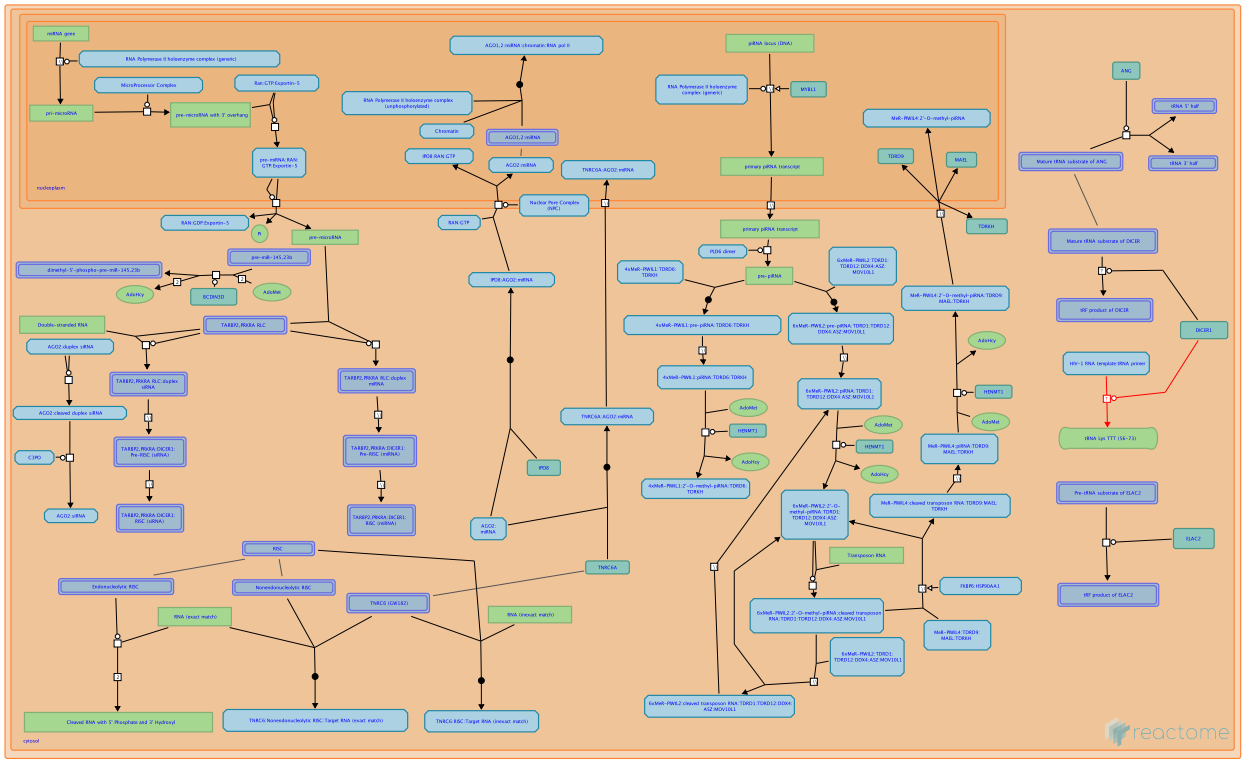
Reactome database release: 77

This document contains 7 pathways (see Table of Contents)

Gene Silencing by RNA ↗

Stable identifier: R-HSA-211000

Compartments: nucleoplasm, nuclear envelope, cytosol



In this module, the biology of various types of regulatory non-coding RNAs are described. Biogenesis and functions of small interfering RNAs (siRNAs) and microRNAs (miRNAs) are annotated. Biogenesis of PIWI-interacting small RNAs (piRNAs) and tRNA-derived small RNAs (tsRNAs) are also annotated.

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Ketting, RF. (2011). microRNA Biogenesis and Function : An overview. *Adv Exp Med Biol, 700*, 1-14. ↗

Carthew, RW., Sontheimer, EJ. (2009). Origins and Mechanisms of miRNAs and siRNAs. *Cell, 136*, 642-55. ↗

Editions

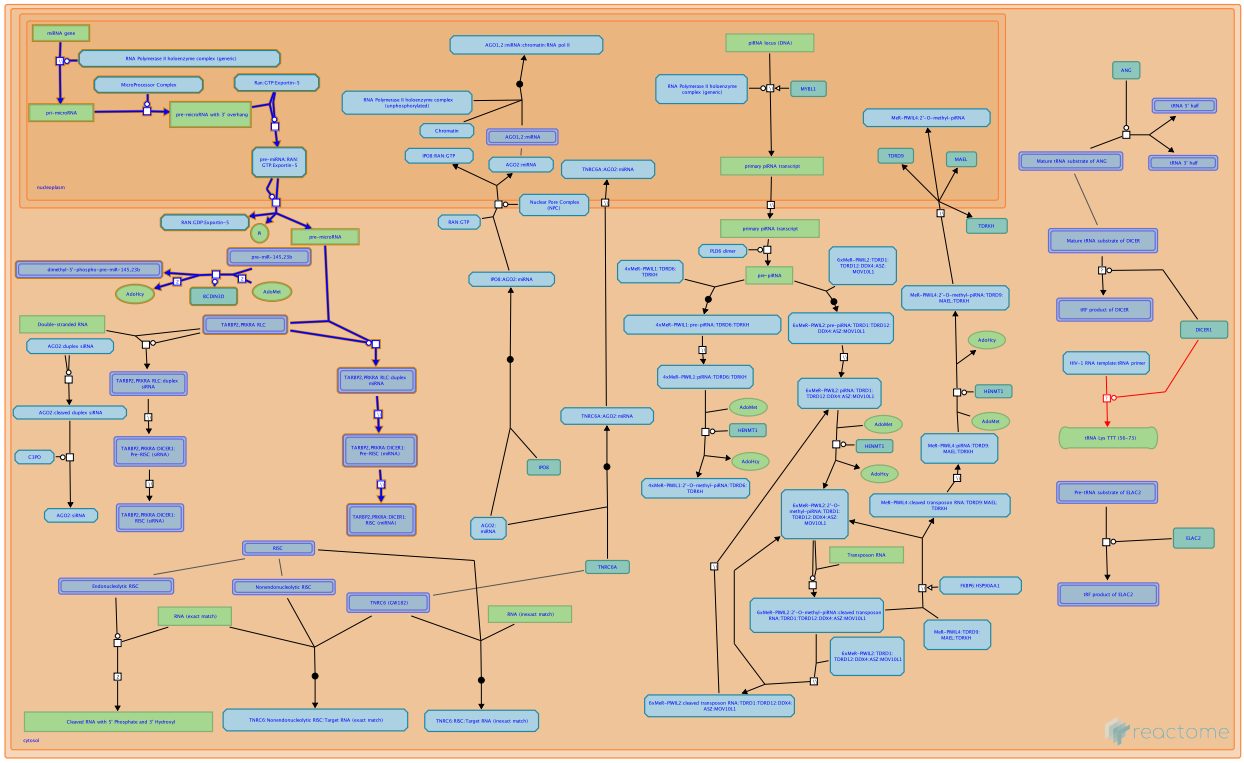
2008-01-23	Authored	Gopinathrao, G.
2008-02-08	Reviewed	Hannon, GJ., Karginov, F.
2009-06-10	Edited	May, B.

MicroRNA (miRNA) biogenesis ↗

Location: Gene Silencing by RNA

Stable identifier: R-HSA-203927

Compartments: nucleoplasm, nuclear envelope, cytosol



Biogenesis of microRNAs (miRNAs) can be summarized in five steps (reviewed in Ketting 2011, Nowotny and Yang 2009, Kim et al. 2009, Chua et al. 2009, Hannon and He 2004):

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 passen-

ger 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.

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Ketting, RF. (2011). microRNA Biogenesis and Function : An overview. *Adv Exp Med Biol, 700*, 1-14. ↗

Editions

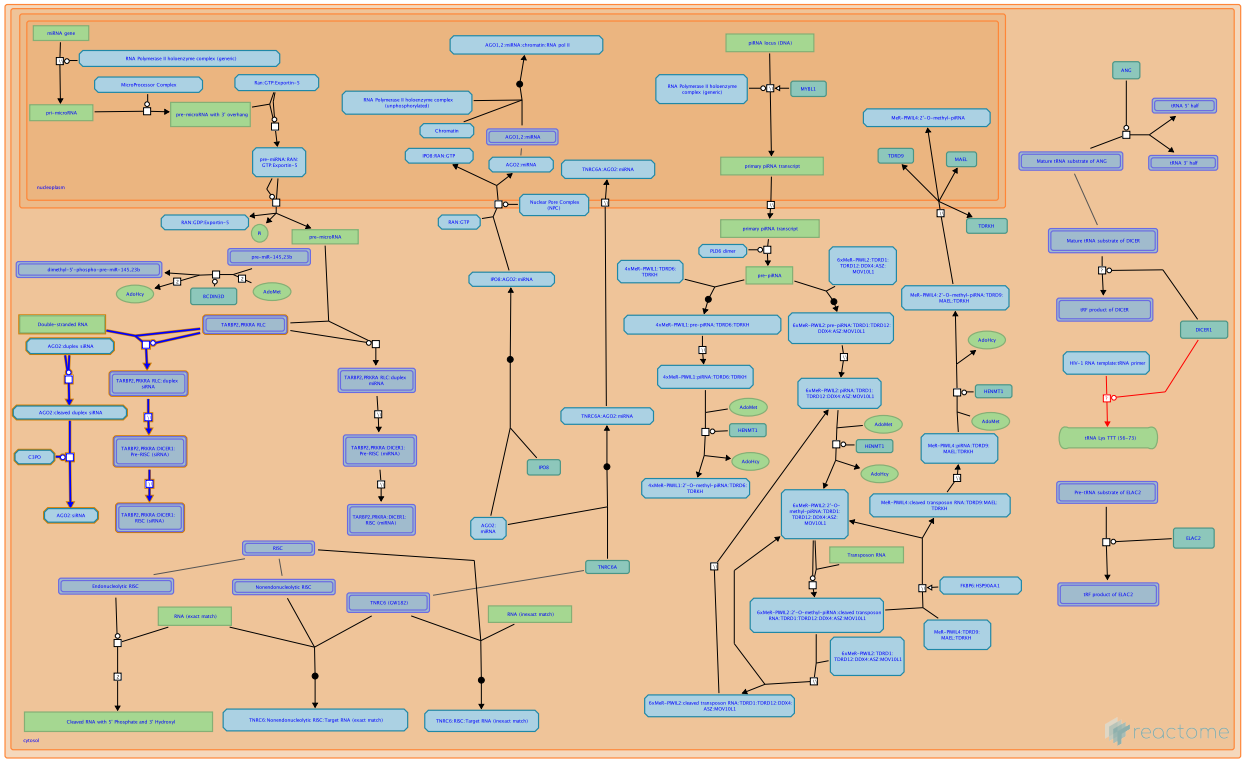
2007-11-19	Authored	Gopinathrao, G., May, B.
2008-02-08	Reviewed	Hannon, GJ., Karginov, F.
2009-03-17	Edited	May, B.

Small interfering RNA (siRNA) biogenesis ↗

Location: Gene Silencing by RNA

Stable identifier: R-HSA-426486

Compartments: cytosol



Small interfering RNAs (siRNAs) are 21-25 nucleotide single-stranded RNAs produced by cleavage of longer double-stranded RNAs by the enzyme DICER1 within the RISC loading complex containing DICER1, an Argonaute protein, and either TARBP2 or PRKRA (PACT). Typically the long double-stranded substrates originate from viruses or repetitive elements in the genome and the two strands of the substrate are exactly complementary.

After cleavage by DICER1 the 21-25 nucleotide double-stranded product is loaded into an Argonuate protein (humans contain 4 Argonautes) and rendered single-stranded by a mechanism that is not well characterized.

siRNA-loaded AGO2 is predominantly located at the cytosolic face of the rough endoplasmic reticulum and has also been observed in the nucleus.

Literature references

Carthew, RW., Sontheimer, EJ. (2009). Origins and Mechanisms of miRNAs and siRNAs. *Cell, 136*, 642-55. ↗

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Editions

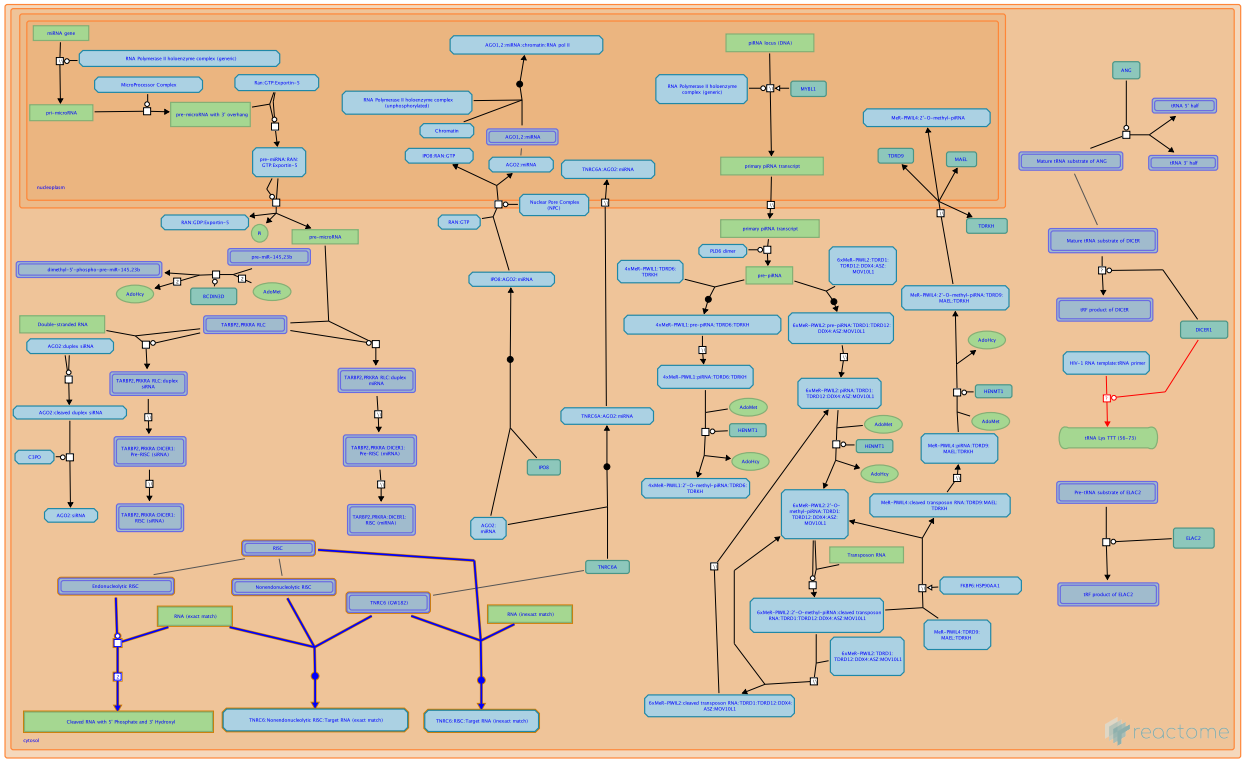
2009-06-10	Authored, Edited	May, B.
2012-02-11	Reviewed	Tomari, Y.

Post-transcriptional silencing by small RNAs ↗

Location: Gene Silencing by RNA

Stable identifier: R-HSA-426496

Compartments: cytosol



Small RNAs act with components of the RNA-induced silencing complex (RISC) to post-transcriptionally repress expression of mRNAs (reviewed in Nowotny and Yang 2009, Chua et al. 2009). Two mechanisms exist: 1) cleavage of target RNAs by complexes containing Argonaute2 (AGO2, EIF2C2) and a guide RNA that exactly matches the target mRNA and 2) inhibition of translation of target RNAs by complexes containing AGO2 and an inexactly matching guide RNA or by complexes containing a nonendonucleolytic Argonaute (AGO1 (EIF2C1), AGO3 (EIF2C3), or AGO4 (EIF2C4)) and a guide RNA of exact or inexact match. Small interfering RNAs (siRNAs) and microRNAs (miRNAs) can serve as guide RNAs in both types of mechanism.

RNAi also appears to direct chromatin modifications that cause transcriptional gene silencing (reviewed in Verdel et al. 2009).

Literature references

Hutvagner, G., Zamore, PD. (2002). A microRNA in a multiple-turnover RNAi enzyme complex. *Science, 297*, 2056-60. ↗

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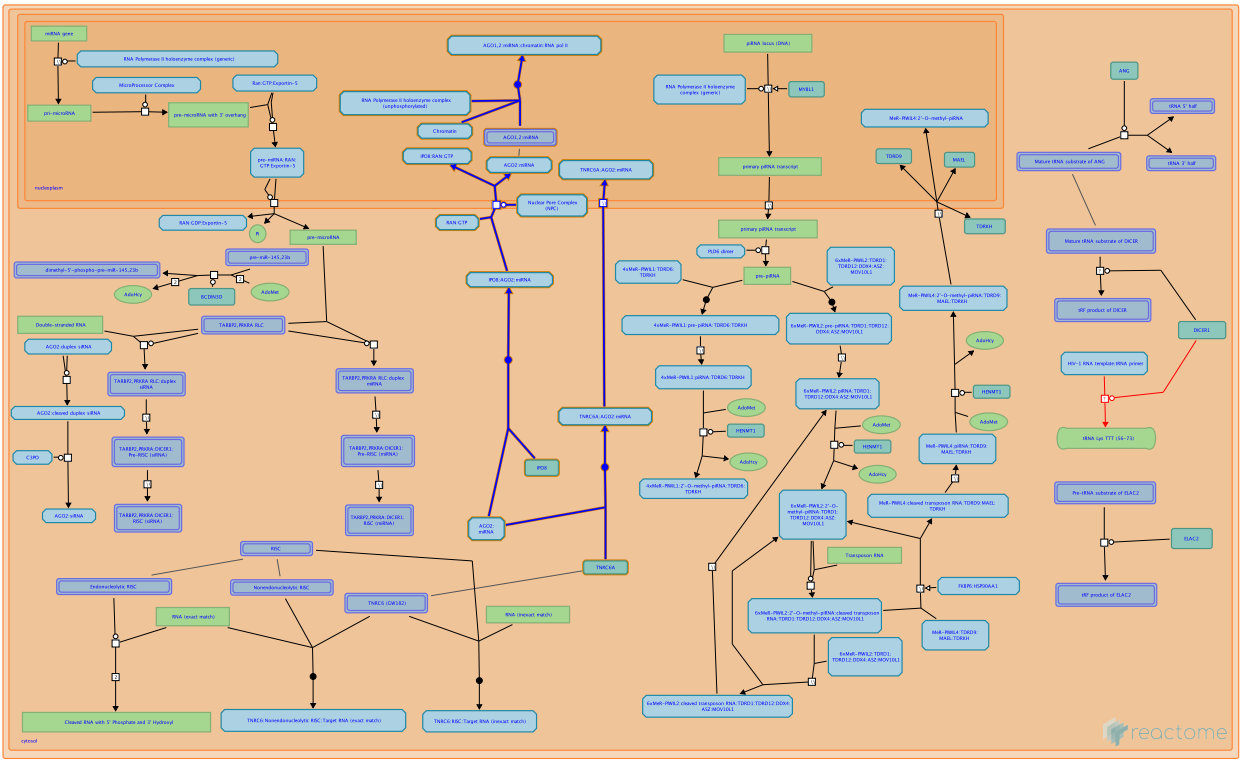
Editions

2009-06-10	Authored, Edited	May, B.
2012-02-11	Reviewed	Tomari, Y.

Transcriptional regulation by small RNAs ↗

Location: Gene Silencing by RNA

Stable identifier: R-HSA-5578749



Recent evidence indicates that small RNAs participate in transcriptional regulation in addition to post-transcriptional silencing. Components of the RNAi machinery (ARGONAUTE1 (AGO1, EIF2C1), AGO2 (EIF2C2), AGO3 (EIF2C3), AGO4 (EIF2C4), TNRC6A, and DICER) are observed associated with microRNAs (miRNAs) in both the cytosol and the nucleus (Robb et al. 2005, Weinmann et al. 2009, Doyle et al. 2013, Nishi et al. 2013, Gagnon et al. 2014). The AGO:miRNA complexes are imported into the nucleus by IMPORTIN-8 (IPO8, IMP8, RANBP8) and also by an unknown importin while associated with the nuclear shuttling protein TNRC6A (reviewed in Schraivogel and Meister 2014).

Within the nucleus, AGO2, TNRC6A, and DICER may associate in a complex (Gagnon et al. 2014). Nuclear AGO1 and AGO2 in complexes with small RNAs are observed to activate transcription (RNA activation, RNAa) or repress transcription (Transcriptional Gene Silencing, TGS) of genes that contain sequences matching the small RNAs (reviewed in Malecova and Morris 2010, Huang and Li 2012, Gagnon and Corey 2012, Huang and Li 2014, Salmanidis et al. 2014, Stroynowska-Czerwinska et al. 2014). TGS is associated with methylation of cytosine in DNA and methylation of histone H3 at lysine-9 and lysine-27 (Castanotto et al. 2005, Suzuki et al. 2005, Kim et al. 2006, Weinberg et al. 2006, Kim et al. 2008, reviewed in Malecova and Morris 2010, Li et al. 2014); RNAa is associated with methylation of histone H3 at lysine-4 (Huang et al. 2012, reviewed in Li et al. 2014). Small RNAs in the nucleus have also been shown to play roles in alternative splicing (Liu et al., 2012, Ameyar-Zazoua et al., 2012) and DNA damage repair (Wei et al., 2012; Francia et al., 2012). Nevertheless, elucidation of the detailed mechanisms of small RNA action requires further research.

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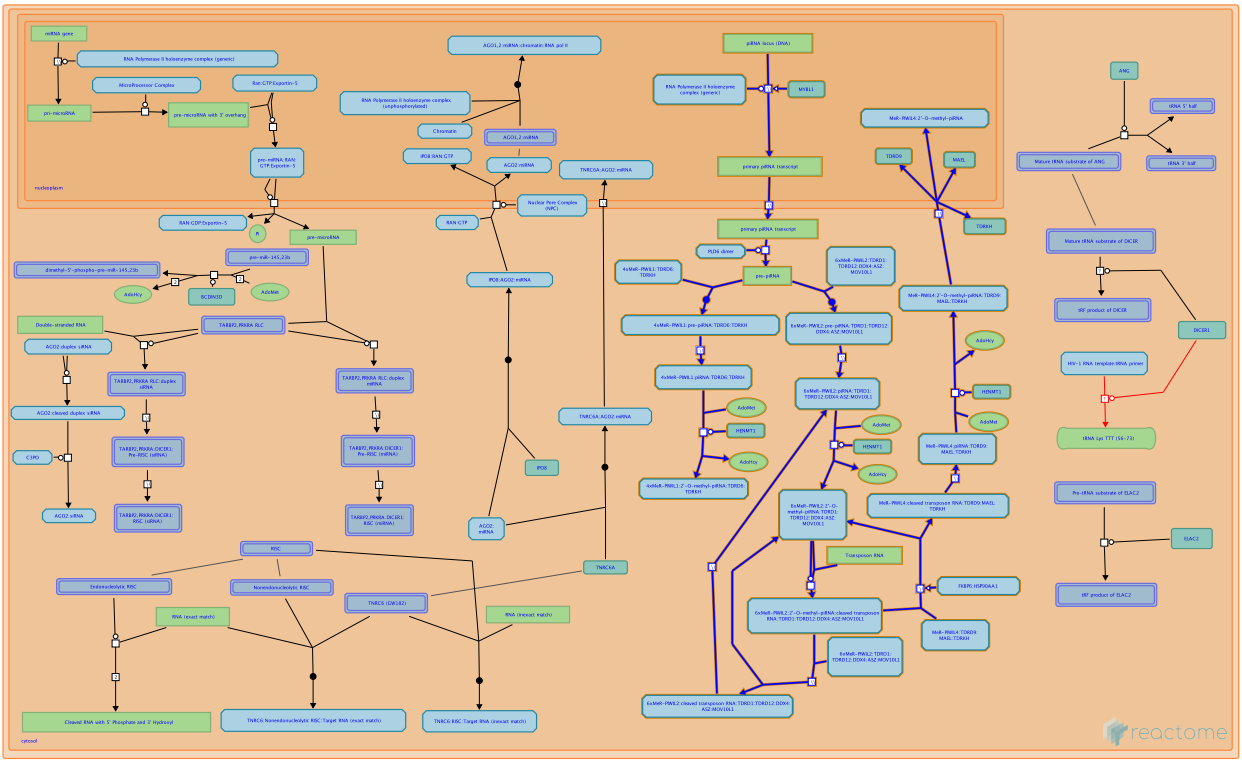
Editions

2014-05-30	Authored, Edited	May, B.
2014-08-25	Reviewed	Li, LC.
2014-09-06	Reviewed	Stroynowska-Czerwinska, A.

PIWI-interacting RNA (piRNA) biogenesis ↗

Location: Gene Silencing by RNA

Stable identifier: R-HSA-5601884



In germ cells of humans and mice, precursors of PIWI-interacting RNAs (piRNAs) are transcribed from a few hundred sequence clusters, as well as individual transposons, intergenic regions, and genes in the genome. These longer transcripts are processed to yield piRNAs of 26-30 nucleotides independently of DICER, the enzyme responsible for microRNAs (miRNAs) and small interfering RNAs (siRNAs) (reviewed in Girard and Hannon 2008, Siomi et al. 2011, Ishizu et al. 2012, Pillai and Chuma 2012, Bortvin 2013, Chuma and Nakano 2013, Sato and Siomi 2013). The initial step in processing long transcripts to piRNAs is cleavage by PLD6 (MitoPLD), which generates the mature 5' end. The cleavage products of PLD6 are bound by either PIWIL1 (HIWI, MIWI) or PIWIL2 (HILI, MILI) in complexes with several other proteins. The 3' end is trimmed by an unknown exonuclease to generate the mature piRNA. PIWIL1:piRNA complexes appear to be involved in post-transcriptional silencing in the cytosol while PIWIL2:piRNA complexes generate further piRNAs from transposon transcripts and other transcripts in the cytosol. Cleavage products from PIWIL2:piRNA may be loaded into either PIWIL2 or PIWIL4 (HIWI2, MIWI2). Loading into PIWIL2 forms a step in a cytosolic amplification loop called the "ping-pong cycle" which yields further PIWIL2:piRNA complexes from cleaved precursor RNAs. Loading into PIWIL4 yields a complex also containing TDRD9 that translocates to the nucleus and directs DNA methylation of cognate loci, causing transcriptional silencing during spermatogenesis. Transcriptional silencing by piRNAs is necessary to limit transposition of endogenous transposons such as L1 elements in the genome.

Literature references

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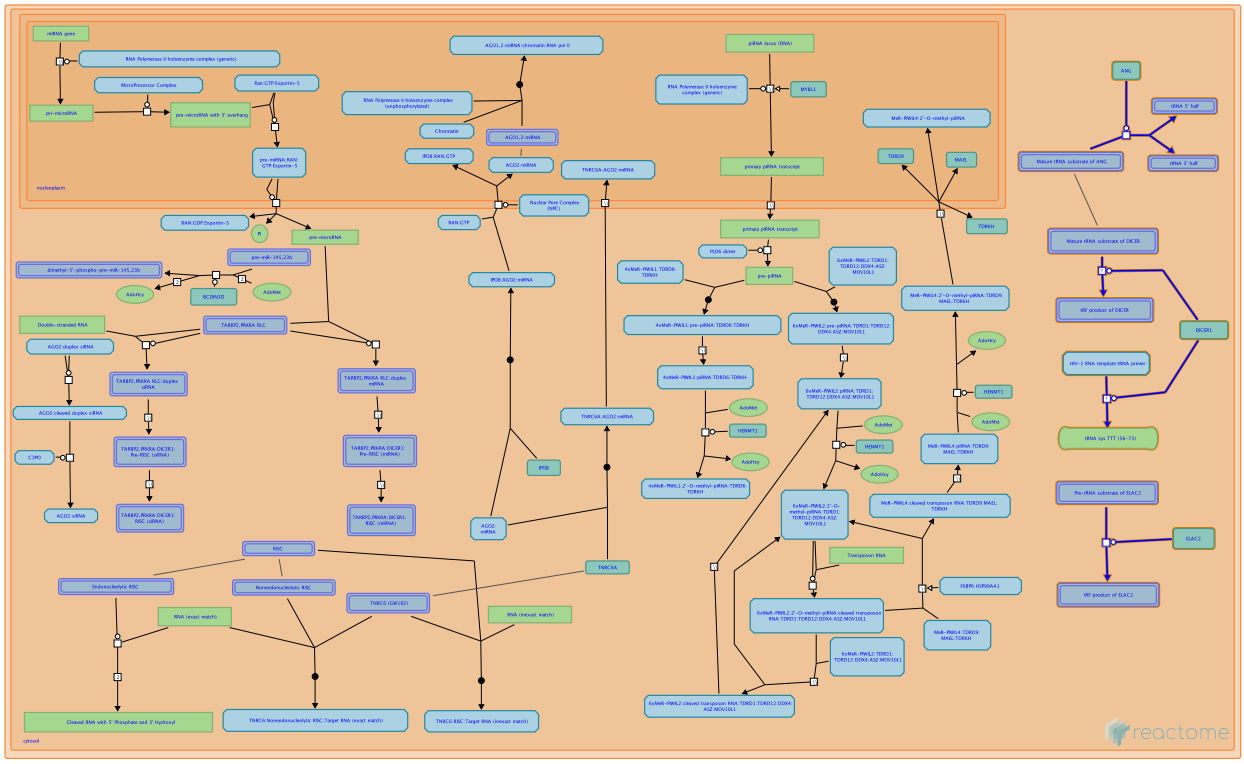
Editions

2014-06-14	Authored, Edited	May, B.
2014-10-25	Reviewed	Saito, K.

tRNA-derived small RNA (tsRNA or tRNA-related fragment, tRF) biogenesis ↗

Location: Gene Silencing by RNA

Stable identifier: R-HSA-9708296



Defined fragments of tRNAs, termed tRNA‐derived small RNAs (tsRNAs), have been observed in particular cell types and in response to biological conditions such as exposure to sex hormone or stresses such as hypoxia, starvation, oxidative stress, and virus infection (reviewed in Keam and Hutvagner 2015, Kumar et al. 2016, Oberbauer and Schaefer 2018, Park et al. 2020, Su et al. 2020, Xie et al. 2020, Zhu et al. 2020). Rather than being the random products of tRNA degradation, tsRNAs appear to be the specific products of ribonucleases. Two categories of tRNA‐derived small RNAs (tsRNAs) have been described: (1) longer (31‐40nt) tsRNAs known as tRNA halves or stress‐induced tsRNAs (tiRNAs) that are produced by single cleavage of tRNAs within or near the anticodon and (2) shorter (15‐30 nt) tsRNAs termed tRNA-related fragments (tRFs) that result from cleavage closer to the 5' or 3' end of the tRNA. tRF-3s are derived from the 3' region of the tRNA, approximately the region from the T loop to the 3' terminus. tRF-5s are derived from the 5' region of the tRNA, approximately the region from the D loop to the 5' terminus. tRF2‐type tRFs (also called internal tRFs) are derived from the central region of the tRNA, approximately the region between the D loop and the T loop and containing the anticodon. tRF-1s, also known as Type II tRFs or 3'U tRFs, are the 3' trailers of particular tRNAs that persist after processing.

In most cases the enzymes responsible for the cleavages are not yet known, however several ribonucleases involved in cleavage of tRNA have been identified: the secreted and endocytosed ribonuclease A family members angiogenin (ANG) and RNase 1; the interferon-induced ribonucleases RNase L, Schlafen 11 (SLFN11) and Schlafen13 (SLFN13 or RNase S13); the cytosolic ribonuclease III‐like (double strand RNA‐specific) enzyme DICER1; and the RNA processing enzyme ELAC2. ANG is secreted, binds receptors on cell membranes, is endocytosed, and translocates to the nucleus. ANG cleaves within the anticodon loop to produce tRNA halves and the cleavage is thought to occur while ANG is transiently located in the cytosol (Lee and Vallee 1989, Saxena et al. 1992, Fu et al. 2009, Yamasaki et al. 2009, Emara et al. 2010, Ivanov et al. 2011). Cleavage by ANG is observed in response to cellular stresses such as starvation (Fu et al. 2009, Yamasaki et al. 2009, Emara et al. 2010, Ivanov et al. 2011). However, ANG knockout cells

continue to produce stress-induced tRNA halves, suggesting that other enzymes are also involved in producing the halves (Su et al. 2019). Similar to ANG as an RNase A member, the secreted endoribonuclease RNase 1 cleaves tRNAs at the anticodon loop in the extracellular space (Nechooshtan et al. 2020).

Interferon-induced RNases can also cleave tRNAs. RNase L is responsive to double stranded RNAs and cleaves at the tRNA anticodon loop (Donovan et al. 2017). Schlafen family members SLFN11 and SLFN13 can also cleave tRNAs (Li et al. 2018, Yang et al. 2018).

DICER1 cleaves double‐stranded regions of tRNAs near the 5' terminus or 3' terminus to produce short tRFs (Cole et al. 2009, Yeung et al. 2009, Maute et al. 2013, Hasler et al. 2016). The mechanism that dissociates the double‐stranded products of DICER1 to yield single‐stranded tRFs may be the same as that for miRNAs, but this has not yet been demonstrated. Furthermore, the bulk of the short tRFs is still detected in DICER1-null cells (Kumar 2014, Kuscu & Kumar et al. 2018), suggesting other unknown factors are involved in their biogenesis. ELAC2 in the cytosol cleaves the 3' trailers of precursors of tRNA Ser TGA, tRNA Ser GTC, and tRNA Asp GTC, and tRNA Asp GTC (Lee et al. 2009). The trailers (also called tRF-1s) then persist in the cytosol (Kumar et al. 2014).

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Emara, MM., Ivanov, P., Hickman, T., Dawra, N., Tisdale, S., Kedersha, N. et al. (2010). Angiogenin-induced tRNA-derived stress-induced RNAs promote stress-induced stress granule assembly. *J Biol Chem, 285*, 10959-68.

Editions

2020-11-27	Authored, Edited	May, B.
2021-02-13	Reviewed	Basso, K.
2021-02-20	Reviewed	Dutta, A., Wilson, B., Su, Z.

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