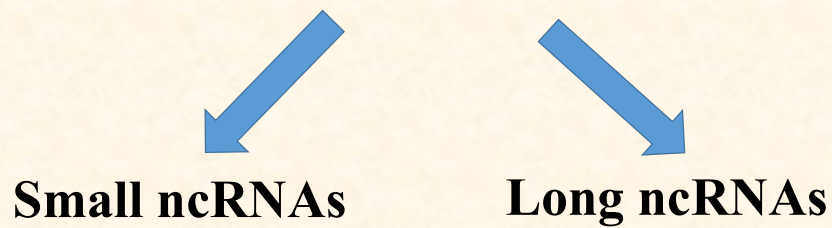


Nekódujúce RNA

Non-coding RNAs

ncRNAs



Arbitrárnny treshold 200 nukleotidov

1

- ncRNA sú funkčné RNA molekuly, ktoré sú transkribované z existujúceho cicavčieho genómu, ale nekódujú žiadny proteín
- 80% ľudského genómu pozostáva z nekódujúcich elementov ako sú malé ncRNA (sncRNAs) a dlhé ncRNAs (lncRNA)

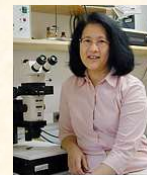
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Table 1 non-coding RNA classification				
	Abbreviation	Full name	Mean size	Function
long ncRNA	rRNA	Ribosomal RNA	~1.9 kb	Essential for protein synthesis
	XIST RNA	X-inactive specific transcript	~17 kb	Chromosome X inactivation
	other lncRNA		> 200 nt	Involved in epigenetic modification and post-transcriptional processing
small ncRNA	miRNA	mature microRNA	18–21 nt	RNA silencing
	siRNA	small interfering RNA	21 nt	RNA silencing, defense against viruses and transposon activity
	piRNA	Piwi interacting RNA	26–30 nt	Regulation of transposon activity and chromatin state
	tRNA	transfer RNA	70–90 nt	translation
	snRNA	small nuclear RNA	100–300 nt	RNA splicing
	snoRNA	small nucleolar RNA	60–300 nt	RNA modification
	scaRNA	small Cajal body RNA	200 nt	RNA modification
	gRNA	guide RNA	40–80 nt	RNA editing
	rasiRNA	repeat associated small interfering RNA	24–27 nt	Orientation of heterochromatin in the formation of centromeres

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Discovery of small RNAs

Rosalind Lee



- The first small RNA:
 - In 1993 Rosalind Lee (Victor Ambros lab) was studying a non-coding gene in *C. elegans*, *lin-4*, that was involved in silencing of another gene, *lin-14*, at the appropriate time in the development of the worm *C. elegans*.
 - Two small transcripts of *lin-4* (22nt and 61nt) were found to be complementary to a sequence in the 3' UTR of *lin-14*.
 - Because *lin-4* encoded no protein, she deduced that it must be these transcripts that are causing the silencing by RNA-RNA interactions.
 - The second small RNA wasn't discovered until 2000!

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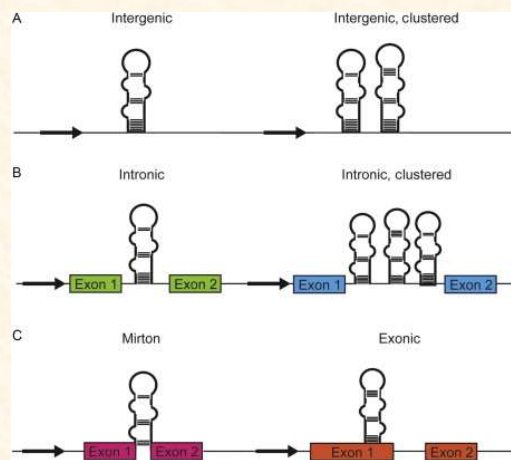
<https://www.nobelprize.org/prizes/medicine/2024/press-release/>

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- malé nekódujúce RNA sú intenzívne študované a sú asociované s RNA interferenčnými dráhami, ktoré spôsobujú silencing špecifických génov vrátane ochrany pred vírusmi

miRNA

- miRNAs are coded by DNA similarly like mRNA
- miRNAs represents 1-5% of the animal genome
- miRNAs are frequently present in clusters forming polycistronin units
- location of miRNA coding DNA sequences can be intronic or intergenic
- miRNAs are frequently located in an intergenic sequence or an antisense strand of genes



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Features of miRNAs

- Hundreds miRNA genes are already identified in human genome.
- Most miRNAs start with a U
- The second 7-mer on the 5' end is known as the "seed."
 - When an miRNAs bind to their targets, the seed sequence has perfect or near-perfect alignment to some part of the target sequence.
 - **Example:** UGAGCUUAGCAG...

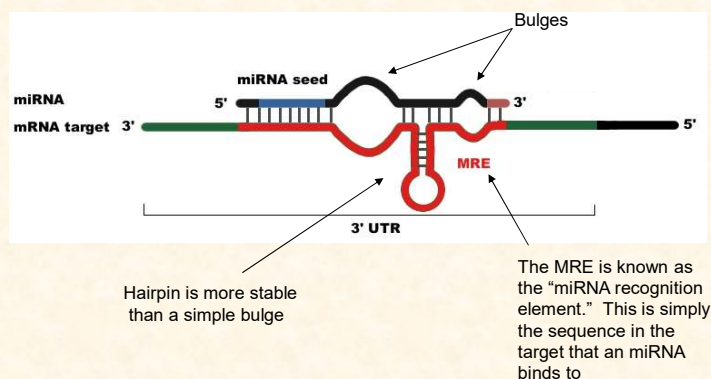
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Features of miRNAs

- Many miRNAs are conserved across species:
 - For half of known human miRNAs, >18% of all occurrences of one of these miRNA seeds are conserved among human, dog, rat, and mouse.
- As a rule, the full sequence of miRNAs is almost never completely complementary to the target sequence.
 - Common to see a loop or bulge after the seed when binding.
 - Loop/bulge is often a hairpin because of stability.
- The site at which miRNAs attack is often in their target's 3' UTR.

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miRNA Binding



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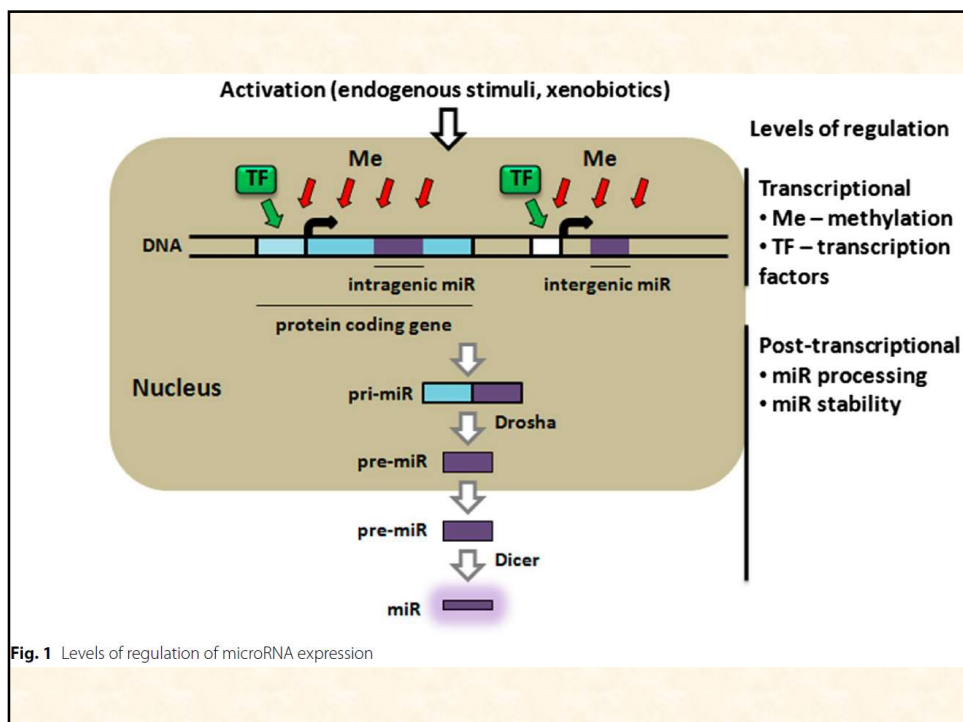
Regulácia transkripcie miRNA

- by their own promoter and regulatory elements
- in human, 50% of miRNAs are located within a gene sequence and they are usually regulated by their host gene promoter
- in such case, miRNAs very often regulate their host genes
- changes in methylation of the regulatory region of miRNAs gene – methylation reduces miRNA expression
- various physiological and pathological stimuli (e.g. steroid hormones, estrogen, tamoxifen, interferon, hypoxia, stress etc.) can affect miRNA expression
- hypoxia is associated with reduced Dicer expression and global miRNA downregulation in the tumor microenvironment
- xenobiotiká, e.g. chemically induced carcinogenesis, aflatoxin B1, alcohol consumption, smoking

Čo je UTR?

[link 2](#)

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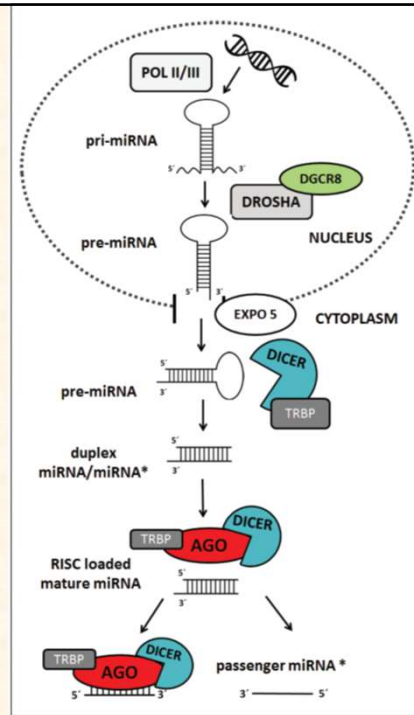
Mechanismus účinku miRNA

- interaction of their “seed” sequences of miRNA with 3′-end and more rarely with 5′-end, of mRNA transcribed from target genes

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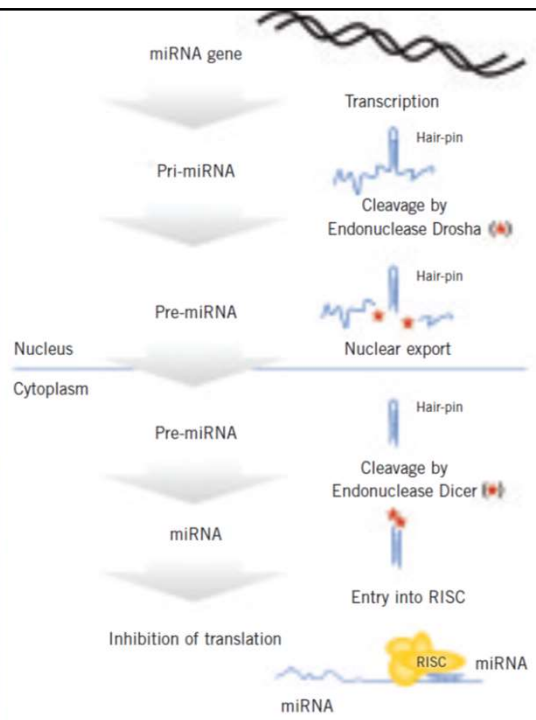
Biosyntéza miRNA

- primary transcripts of miRNA (primary precursor miRNA - pri-miRNA) contain a 5' - 7-methyl guanosine cap and poly(A) tail
- are much longer than mature miRNAs and contain hairpin structure
- after pri-miRNA synthesis they are processed by Microprocesor complex in the nucleus
- microprocesor complex consists from RNaseIII enzyme called Drosha that cleaves pri-miRNA in complex with DiGeorge syndrome critical region gene 8 (DGCR8) to generate premature miRNA (pre-miRNA)
- pre-miRNA is ~70nt long sequence with hairpin structure and a ~30nt stem consisting from dsRNA with 2-3nt overhang on the 3'end

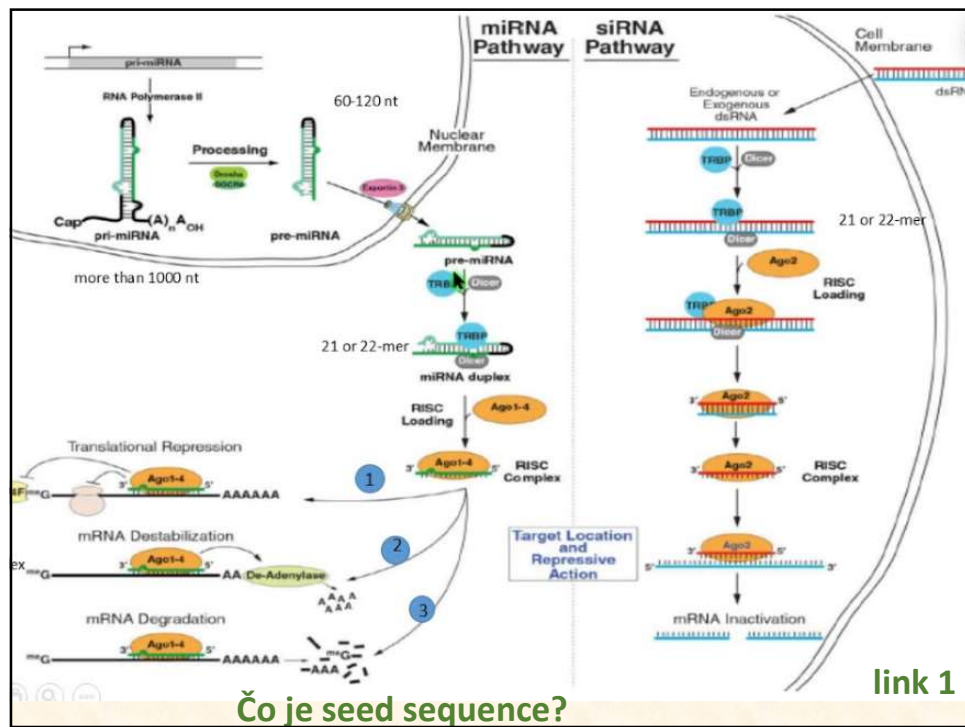


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- after translocation of pre-miRNA into the cytoplasm it is cleaved by RNaseIII Dicer
- mature miRNA is a double stranded RNA helix with 2-3' overhangs at both 3'ends



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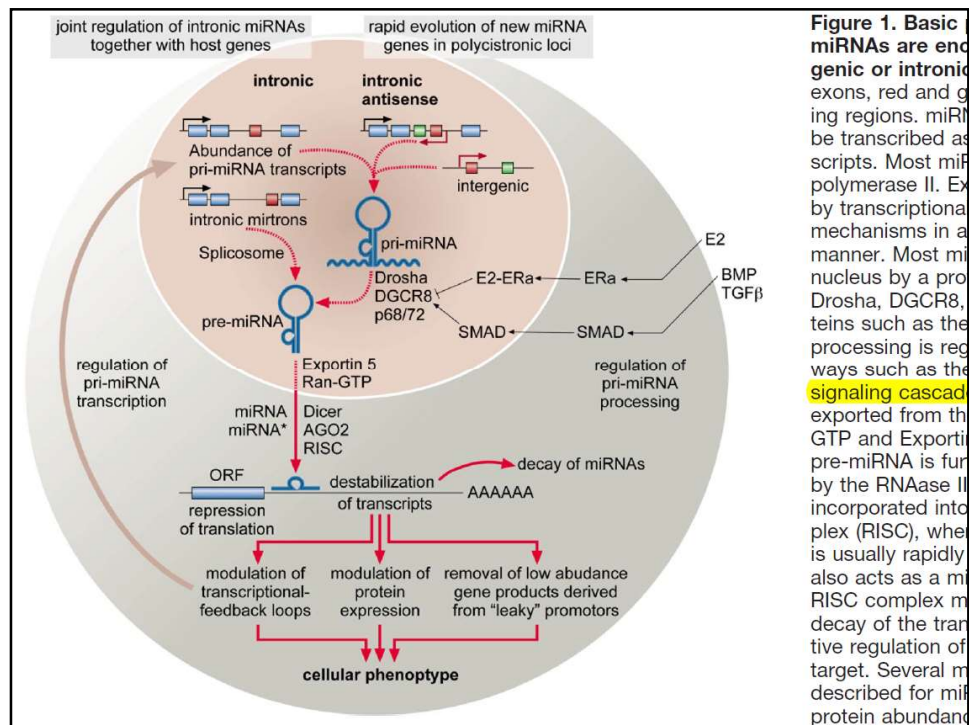


Figure 1. Basic miRNAs are endogenous or intronic exons, red and green regions. miRNAs can be transcribed as introns or exons. Most miRNAs are transcribed by RNA polymerase II. Exportin 5 transports pre-miRNA to the cytoplasm by transcriptional mechanisms in a manner. Most miRNAs are processed by Dicer into 21 or 22-mer. RISC Loading with Ago 2 forms the RISC Complex. Target Location and Repressive Action includes mRNA inactivation.

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Table 1 miRNA/mRNA interactions with strong experimental evidence supporting role in physiological function				
	microRNA	Validated targets	Function	Reference
Cancer	let-7	RAS	↓ regulation of oncogene	Johnson et al. 2005
	miR-16	VEGF	regulation of physiological or pathological angiogenesis	Ye et al. 2008
	miR-17	RND3	↓ regulation of tumor suppressor gene	Luo et al. 2012
	miR-18a	ATM	regulation of apoptosis and DNA repair	Wu et al. 2013
	miR-21	PDCD4 PTEN RECK	↓ regulation of tumor suppressor genes	Asangani et al. 2008; Xiong et al. 2013; Zhang et al. 2008
	miR-21	SPRY TIAM1	↓ regulation of oncogene expression	Sarkar et al. 2010; Cottonham et al. 2010
	miR-31	RASA1 LATS2 PPP2R2A RhoBTB1	↓ regulation of tumor suppressor genes	Sun et al. 2013; Liu et al. 2010b; Xu et al. 2013
	miR-31	HDAC2 CDK2	regulation of cell cycle and metastases in liver	Kim et al. 2015
	miR-34	E2F3	regulation of tumor proliferation and apoptosis	Welch et al. 2007; Yang et al. 2015
	miR-214			
AGTR1 – angiotensin II receptor type 1; AR – androgen receptor; ATM – ATM serine/threonine kinase; CDK – cyclin-dependent kinase; CRY1 – cryptochrome 1; ESR1 – estrogen receptor 1; E2F3 – E2F transcription factor 3; IRS-1 – insulin like growth factor 1 receptor; LARS – large tumor suppressor 2; PDCD4 – programmed cell death 4; PER – period, PPP2R2A – protein phosphatase 2 regulatory subunit B, alpha; PTEN – phosphatase and tensin homolog; RAS – retrovirus associated sequence oncogene family; RASA1 – RAS p21 protein activator 1; RasGAP – Ras GTPase activating protein; RHOBTB1 – Rho-related BTB domain containing 1; RECK – reversion inducing cysteine rich protein with kazal motifs; RND3 – Rho family GTPase 3; SPRY – sprouty RTK signaling antagonist 2; THRAP – thyroid hormone receptor (TR) coregulator TR Associated protein 1; TIAM – T-cell lymphoma				

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Hormonal regulation of cancer	miR-18a	ESR1	control of proliferation of hepatocellular carcinoma cells	Liu et al. 2009
	miR-22	ESR1	control of breast cancer proliferation	Xiong et al. 2010
	miR-124	AR	regulation of various type of tumor growth	Shi et al. 2013
	miR-145	IRS1	inhibition of tumor growth (GIT cancer)	Shi et al. 2007
	miR-206	ESR1	control of breast cancer proliferation	Adams et al. 2007
Cardiovascular system	miR-1	RasGAP CDK9 fibronectin	development of cardiac hypertrophy	Sayed et al. 2007
	miR-126	VCAM1	regulation of vascular inflammation	Harris et al. 2008
	miR-155	AGTR1	regulation of renin-angiotensin system	Zheng et al. 2010
	mirR-208	THRAP	regulation of stress dependent cardiac growth	van Rooij and Olson 2009
AGTR1 – angiotensin II receptor type 1; AR – androgen receptor; ATM – ATM serine/threonine kinase; CDK – cyclin-dependent kinase; CRY1 – cryptochrome 1; ESR1 – estrogen receptor 1; E2F3 – E2F transcription factor 3; IRS-1 – insulin like growth factor 1 receptor; LARS – large tumor suppressor 2; PDCD4 – programmed cell death 4; PER – period, PPP2R2A – protein phosphatase 2 regulatory subunit B, alpha; PTEN – phosphatase and tensin homolog; RAS – retrovirus associated sequence oncogene family; RASA1 – RAS p21 protein activator 1; RasGAP – Ras GTPase activating protein; RHOBTB1 – Rho-related BTB domain containing 1; RECK – reversion inducing cysteine rich protein with kazal motifs; RND3 – Rho family GTPase 3; SPRY – sprouty RTK signaling antagonist 2; THRAP – thyroid hormone receptor (TR) coregulator TR Associated protein 1; TIAM – T-cell lymphoma				

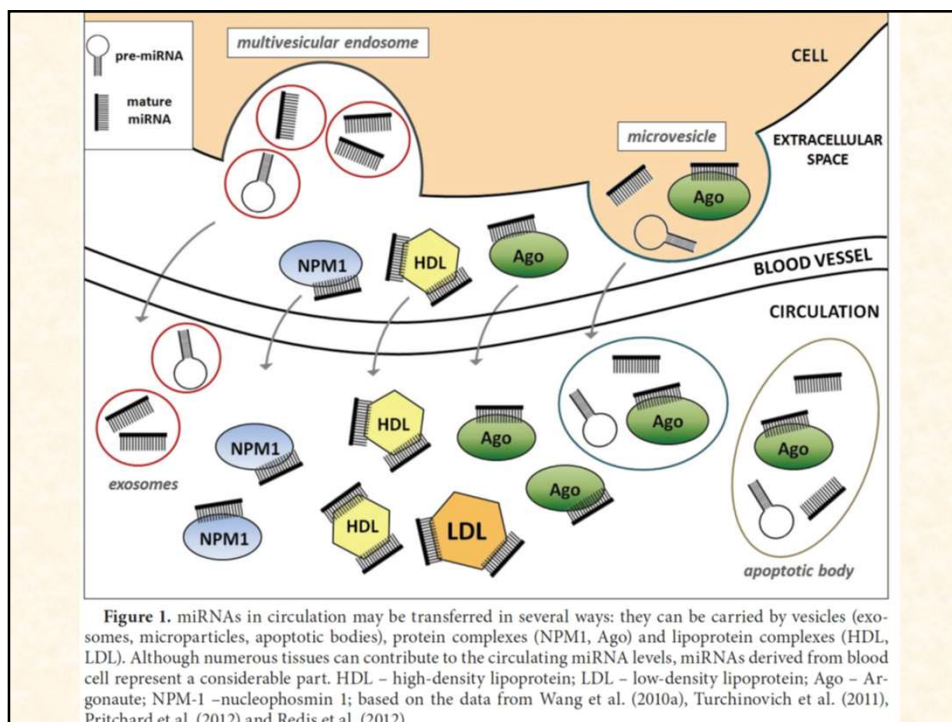
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Circadian rhythms	miR-141	CLOCK	regulation of circadian rhythms	Kiriakidou et al. 2004
	miR-152 miR-494	BMAL1	regulation of circadian rhythms	Shende et al. 2011
	miR-185	CRY1	regulation of circadian rhythms	Lee et al. 2013
	miR-192/194 cluster	PER (1-3)	regulation of circadian rhythms	Nagel et al. 2009

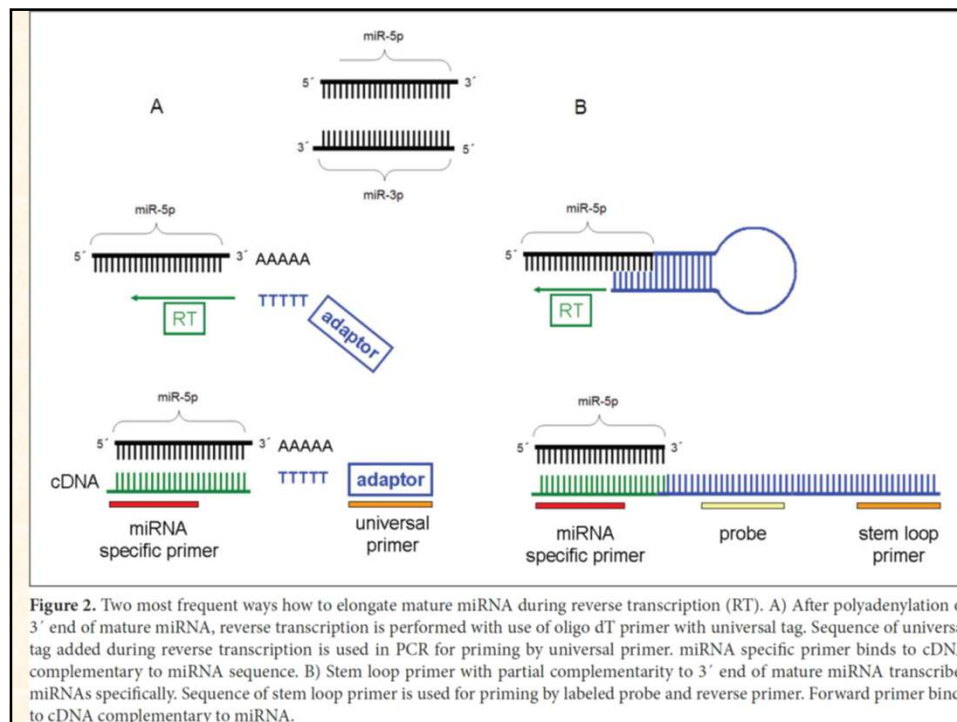
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Čo je funkciou enzýmu drosha? link 3

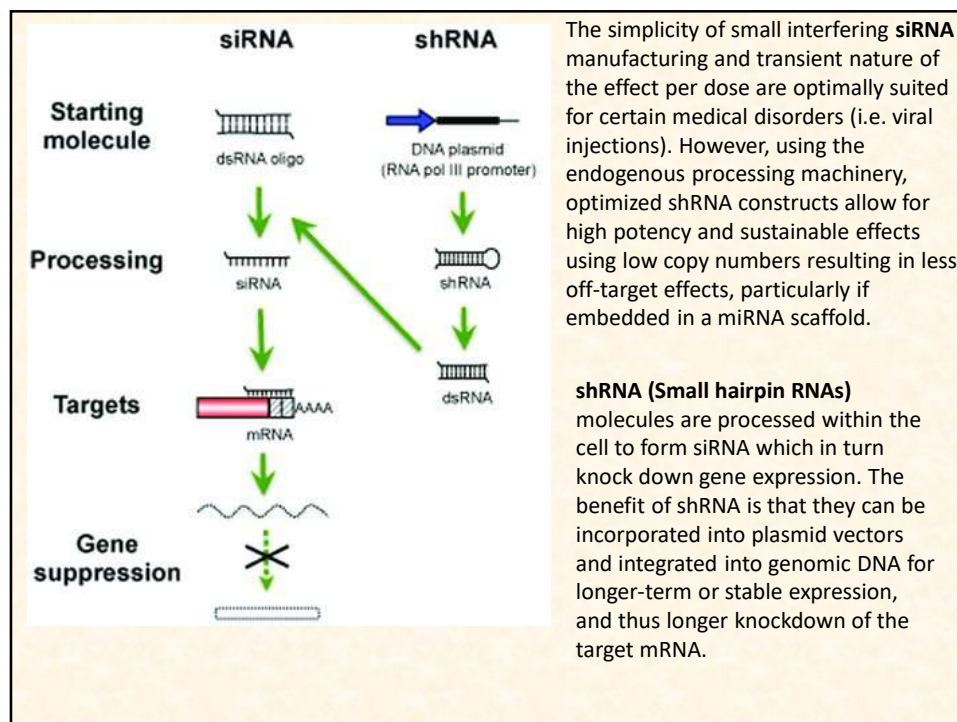
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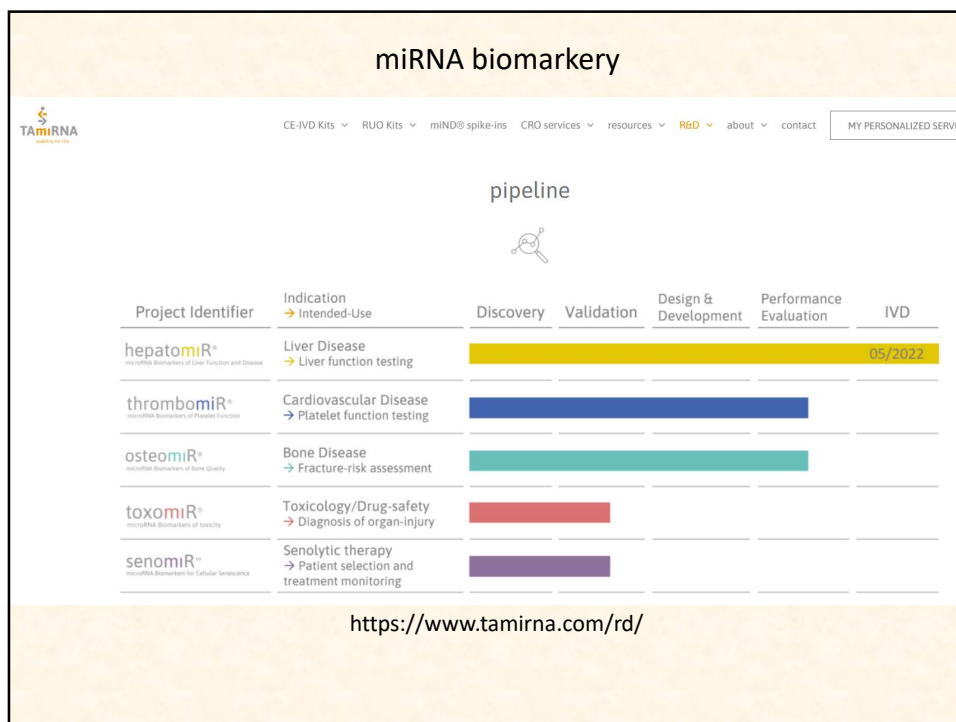
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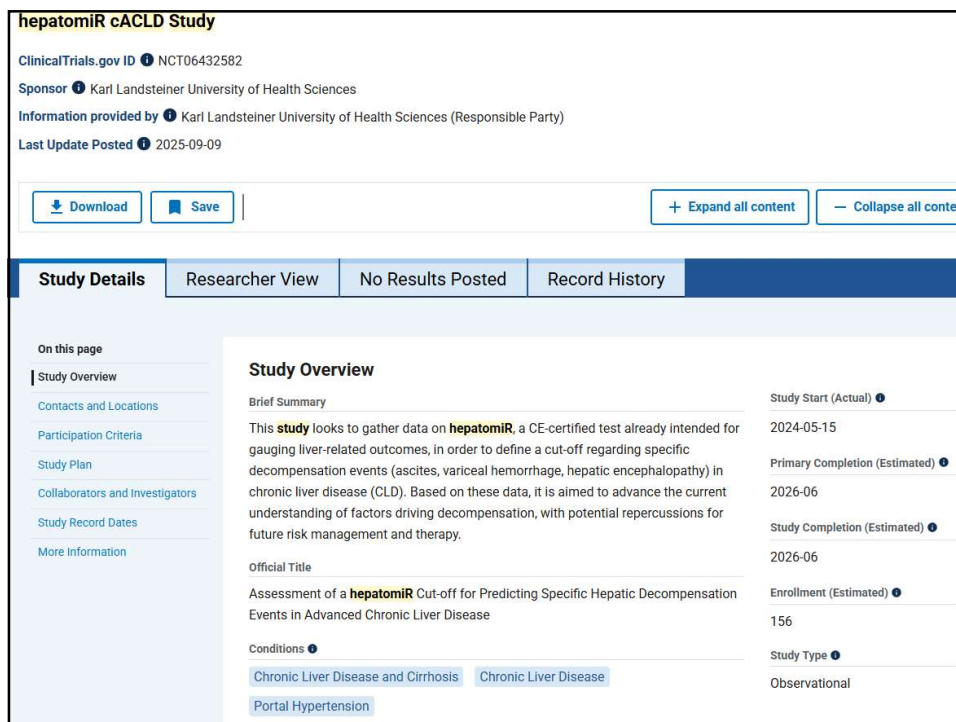
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Table 1. miRNA-based therapeutics in clinical trials.

miRNA-Based Therapeutics					
Company	Name	Therapeutic Agent	Delivery System	Target Disease	Stage in Drug Development Pipeline
Santaris Pharma/Roche	Miravirsen	AntimiR-122	LNA antagomiR	Hepatitis C; Chronic hepatitis C	Phase II clinical trials (NCT02452814; NCT2508090)
	RG-101	AntimiR-122	GaLNac-conjugated antagomiR	Chronic hepatitis C	Phase II clinical trials (discontinued)
Regulus Therapeutics	RG-125	AntimiR-103/107	GaLNac-conjugated antagomiR	Diabetic non-alcoholic steatohepatitis	Phase II (discontinued)
	RG-012	AntimiR-21	NA	Hereditary nephritis	Phase II (NCT02855268)
	RGLS4326	AntimiR-17	NA	Autosomal dominant	Phase I (on hold)

Čo je funkciou enzýmu dicer?

[Link 4](#)

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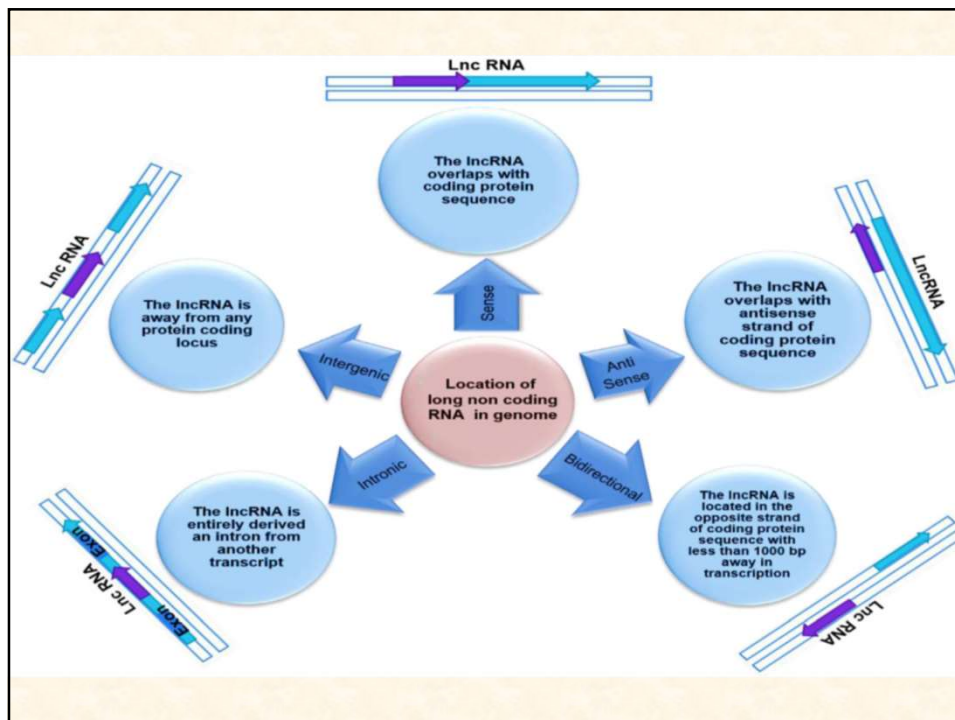
polycystic kidney disease					
miRagen Therapeutics	MRG-106	AntimiR-155	LNA-modified antisense inhibitor	CTCL, mycosis fungoides subtype; CLL; DLBCL; ATLL	Phase II (NCT03713320; NCT03837457); Phase I (NCT02580552)
	MRG-107	AntimiR-155	NA	ALS; cardiac disorders; retinal disorders	Pre-Clinical
	MRG-110	AntimiR-92	LNA antagomiR	Wounds	Phase I (NCT03603431)
	MRG-201	miR-29 mimic	Cholesterol-conjugated miRNA duplex	Keloid; fibrosis	Phase II (NCT03601052); Phase I (NCT02603224)
EnGeneIC	MesomiR-1	miR-16 mimic	EnGeneIC Dream Vector	Malignant pleural mesothelioma; non-small-cell lung cancer	Phase I (NCT02369198)
Mirna Therapeutics Inc.	MRX-34	miR-34 mimic	dsRNA liposomal nanoparticle	Solid tumours; haematological malignancies	Phase I (terminated)
LNA: locked nucleic acid; GaLNac: N-acetylgalactosamine; CTCL: cutaneous T cell lymphoma; ATLL: adult T-cell leukaemia lymphoma; CLL: chronic lymphocytic leukaemia; DLBCL: diffuse large B-cell lymphoma [activated B-cell (ABC) subtype]; ALS: amyloid lateral sclerosis.					

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lncRNA

- ncRNA ovplyvňujú génovú expresu
- lncRNAs pôsobia ako post-transkripčný regulátor, ovplyvňujú DNA metyláciu a remodeláciu chromatinu čím významne participujú na epigenetických reguláciách
- expresia lncRNAs je regulovaná na tkanivovo špecifickej úrovni a v závislosti od vývinového štádia
- deregulácia lncRNA býva spojená s tumorigenézou

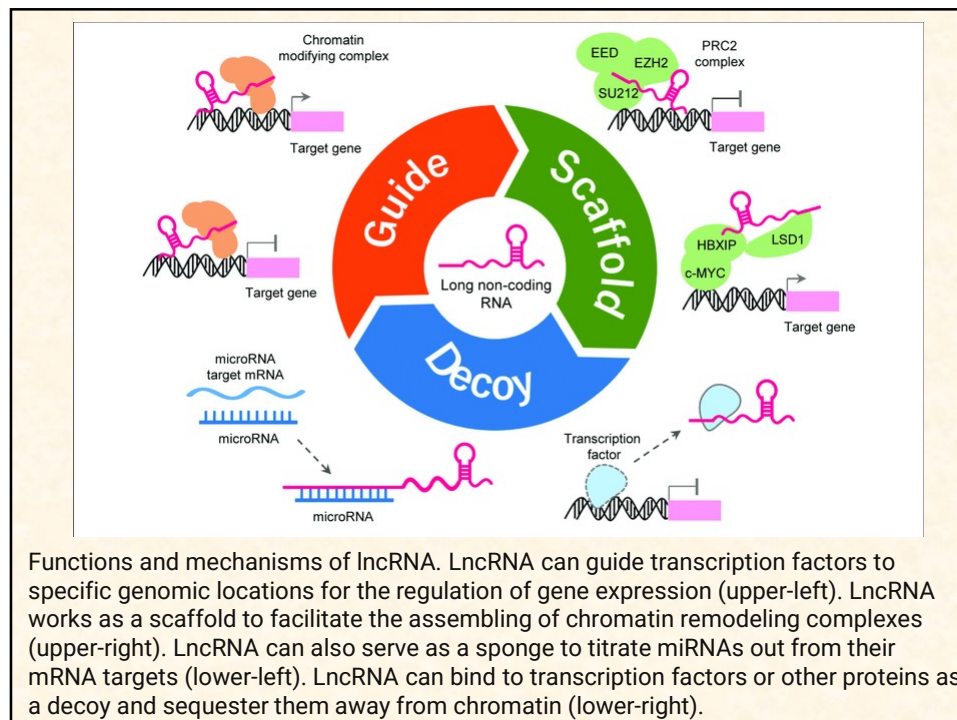
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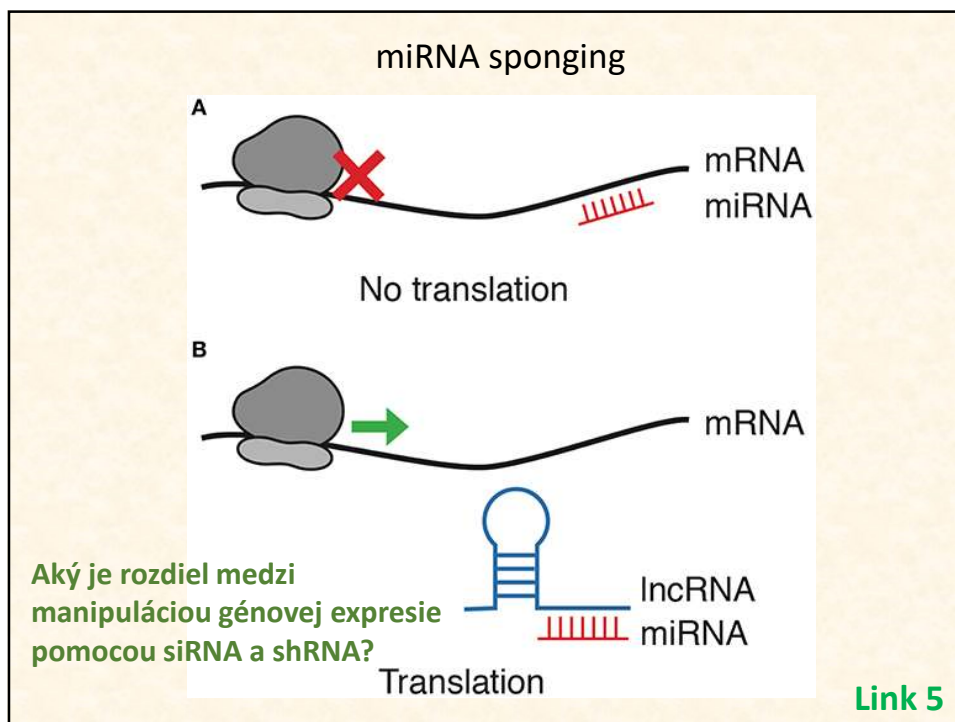
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- podobne ako kódujúca RNA lncRNAs sekvencia obsahuje epigenetické značky indikujúce, že môžu byť exprimované viacerými spôsobmi
- obsahujú intróny umožňujúce alternatívny splicing
- lncRNAs môžu byť polyadenylované aj bez polyA konca a preto sa nazývajú bimorfické
- môžu vykazovať široké spektrum sekundárnych a terciálnych štruktúr
- **nazývajú sa podľa funkcií, ktoré vykonávajú:**
 - intergenic (lincRNA)
 - natural antisense transcripts (NATs)
 - enhancer RNA (eRNA), circular RNA (circRNA)
 - promoter associated long RNA (pRNA), etc.
- lncRNAs môžu pôsobiť na rôznych úrovniach génovej expresie
- vykazujú funkčnú variabilitu, môžu byť prepojené s mnohými substrátmi veľmi špecificky
- lncRNAs sa prekrývajú s kódujúcimi génmi, prekryv môže byť čiastočný, alebo úplný

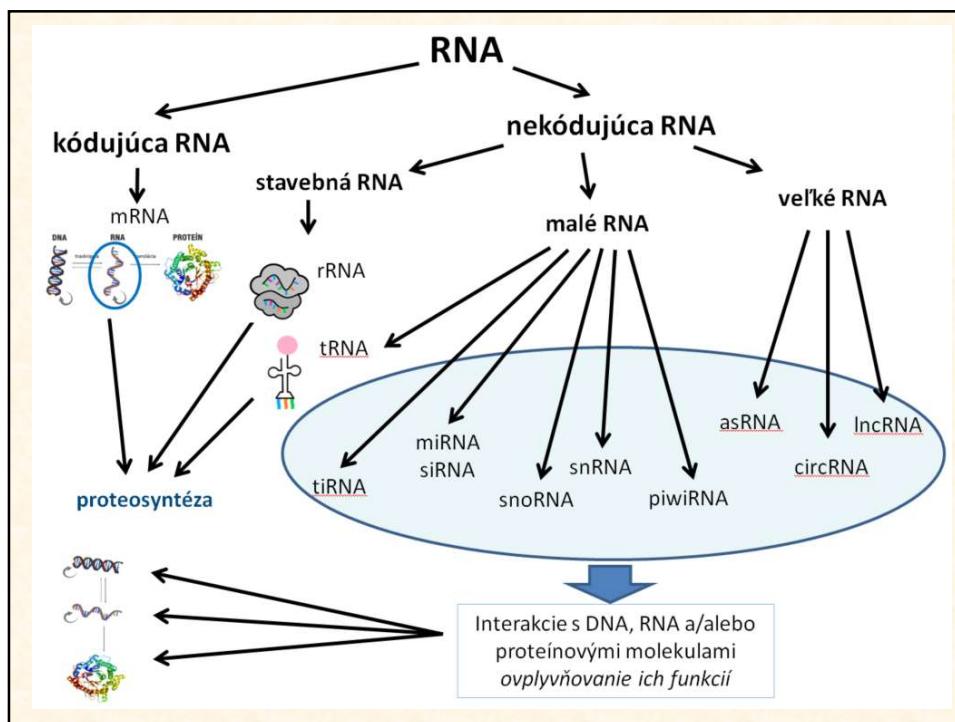
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Ďakujem za pozornosť