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جمعية القلب السعودية
Saudi Heart Association

Gene-Silencing Technology and Potential Applications

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Bellevue Medical Center
Associate Prof. Lebanese University



INTRODUCTION

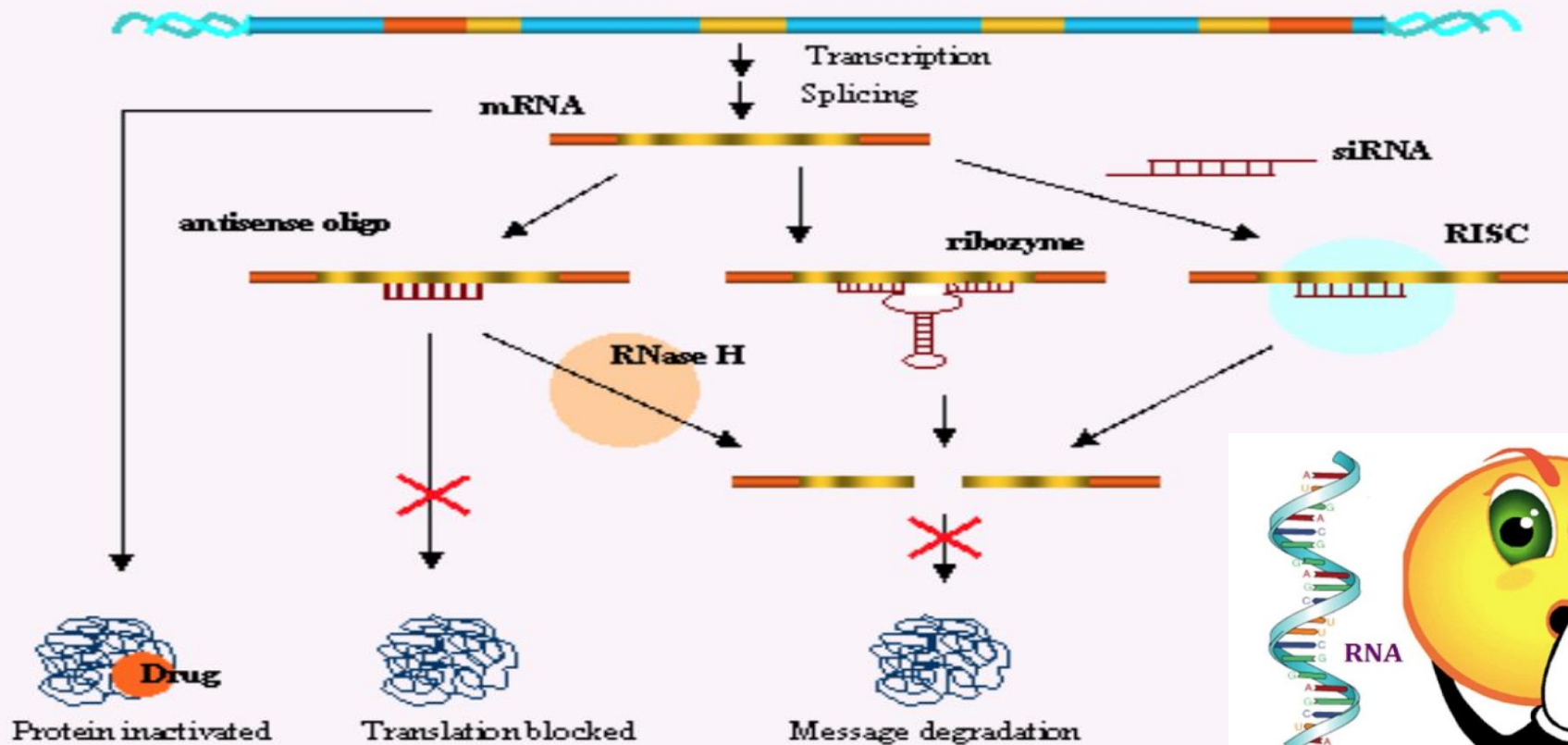
- Gene silencing is a technique that aims to reduce or eliminate the production of a protein from its corresponding gene.
- It generally describes the “**switching off**” of a gene by a mechanism other than genetic modification.
- It occurs when RNA is unable to make a protein during translation.
- Gene silencing is the same as gene **knock down** but is totally different from gene **knock out**.



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Genomic DNA



RNA



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There are so many approaches for gene silencing

- Gene Knockout
- Gene Knockdown
- Gene silencing and degradation of gene using RNA technology
 - Antisense RNA Technology
 - RNAi Technology



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Types of Gene silencing

- Genes are regulated at either the transcriptional level or post-transcriptional level, therefore silencing can be induced either at transcriptional level or post-transcriptional level.
- There are mainly two types of gene silencing
 1. Transcriptional gene silencing
 2. Post transcriptional gene silencing

Transcriptional gene silencing	Post transcriptional gene silencing
1. Genomic Imprinting	1. Antisense RNA technology
2. Paramutation	2. RNAi technology
3. Transposon silencing	- mi RNA
4. Transgene silencing	- sh RNA
5. Position effect	- si RNA
6. RNA-directed DNA methylation	

26/9/2016

7

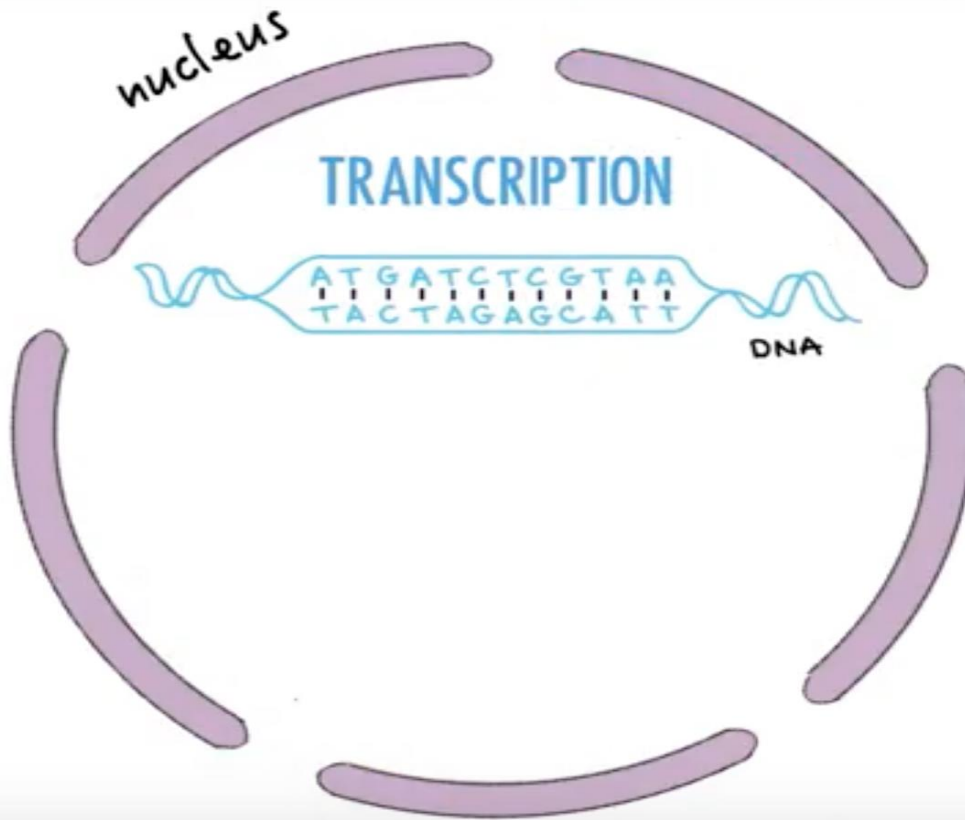


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PROTEIN SYNTHESIS

TRANSLATION

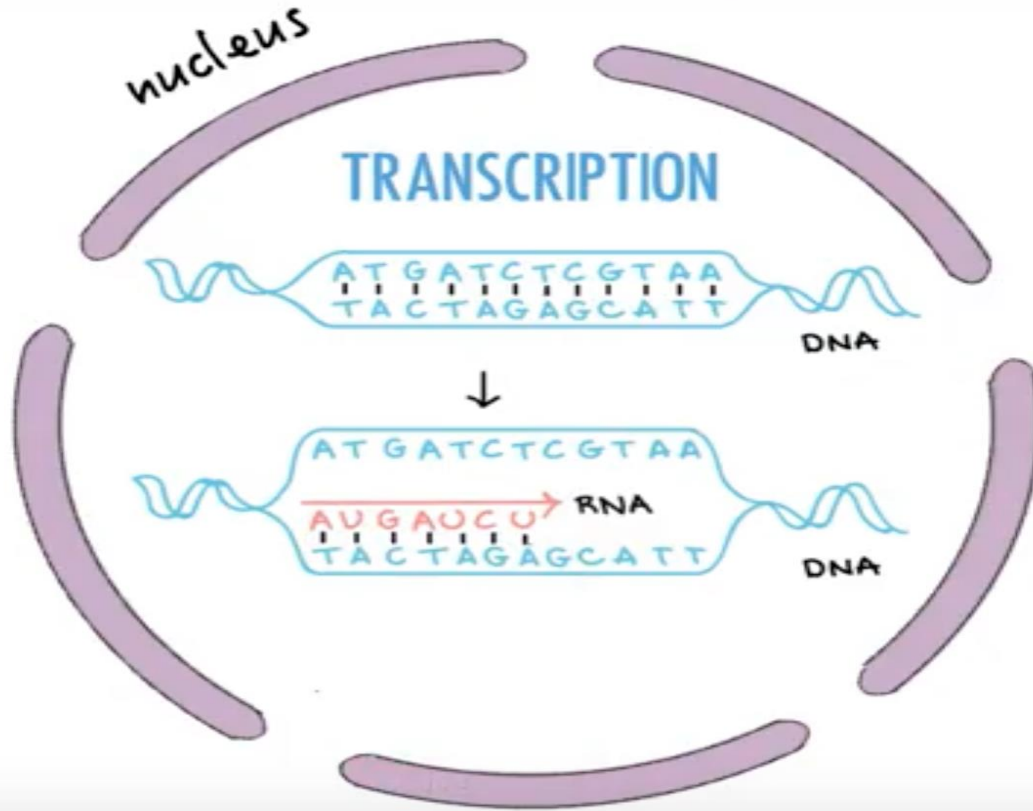


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PROTEIN SYNTHESIS

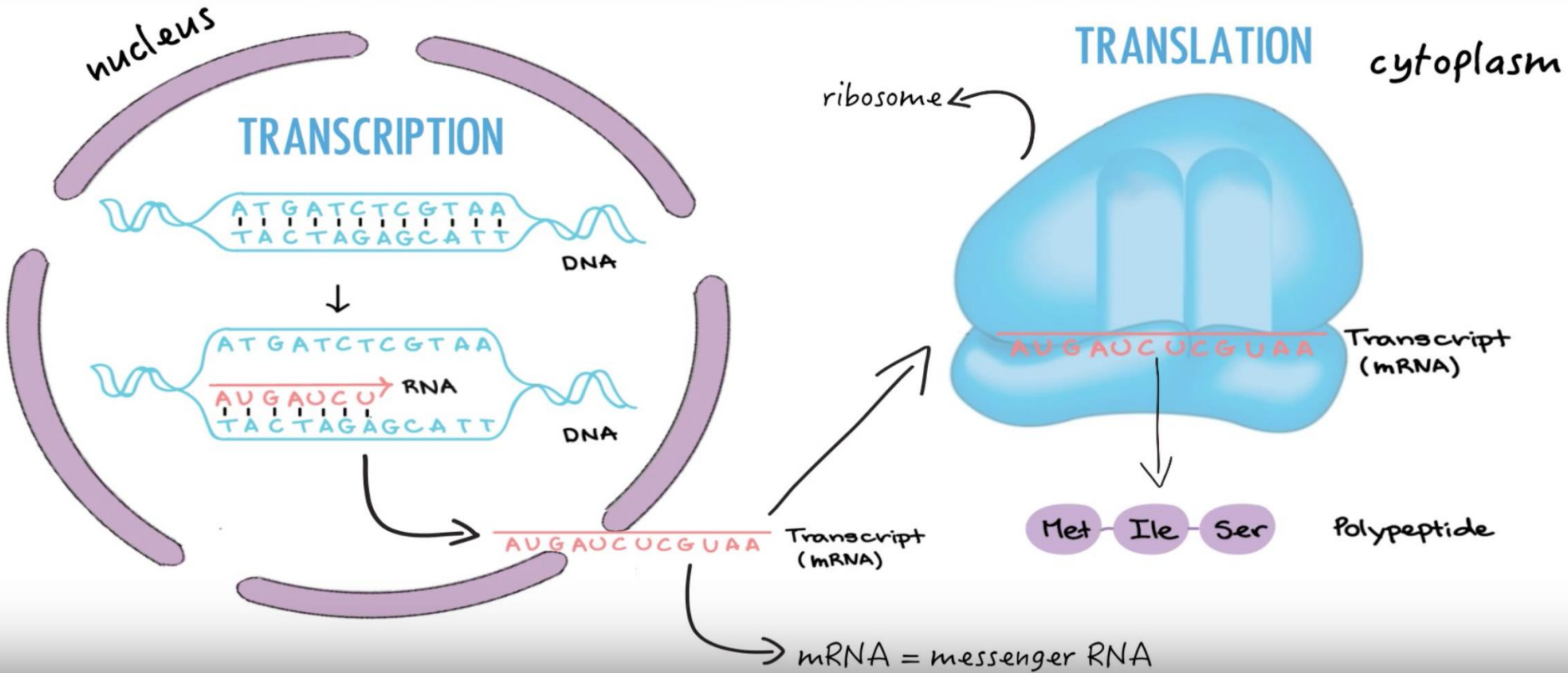
TRANSLATION



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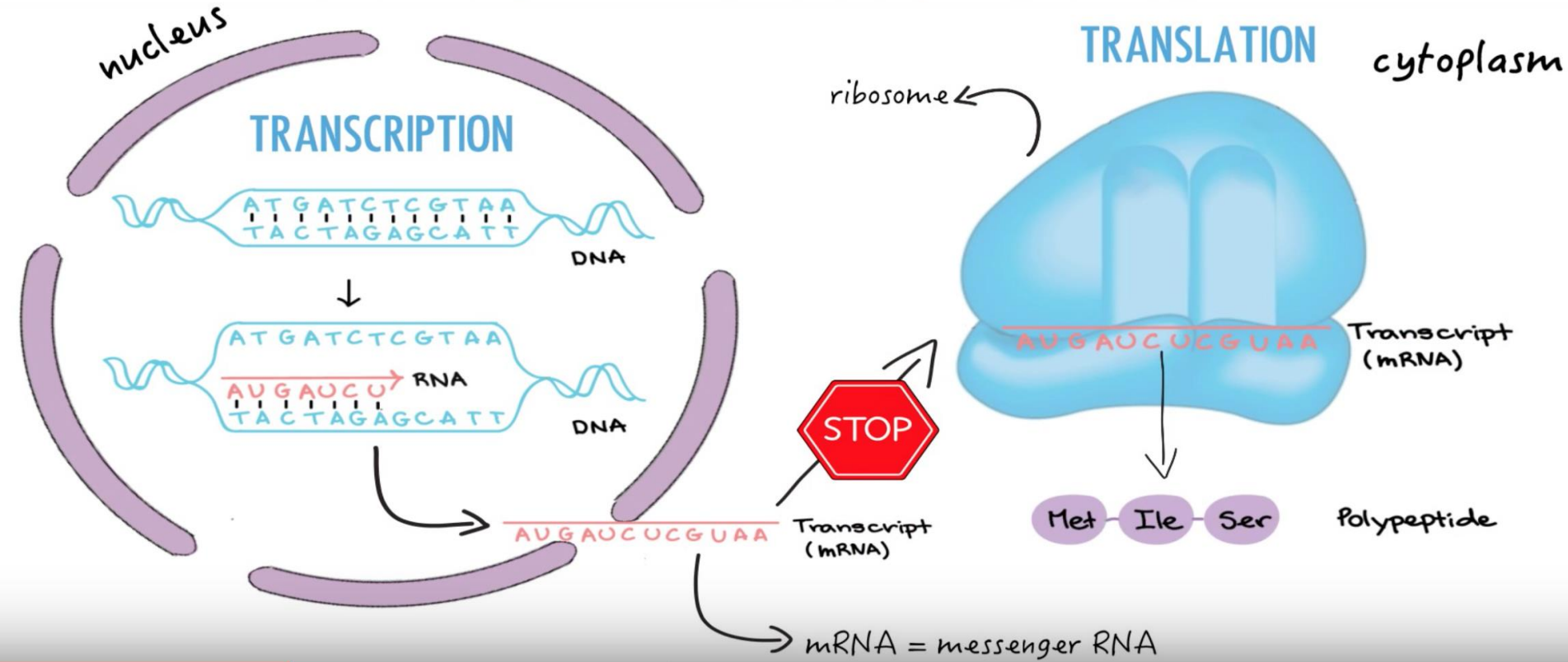
PROTEIN SYNTHESIS



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PROTEIN SYNTHESIS



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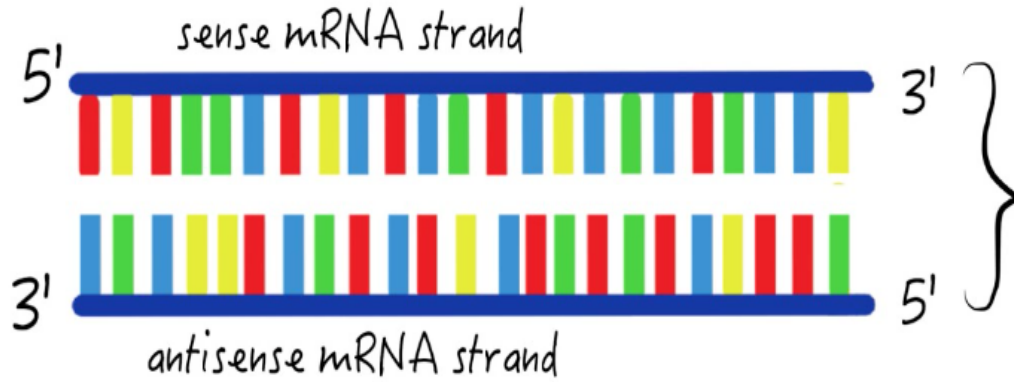
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Sense mRNA Strand



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DOUBLE STRANDED RNA
(dsRNA)



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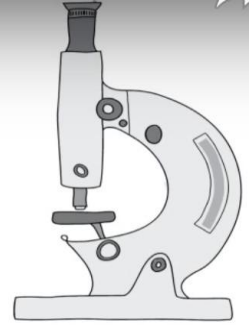




Andrew Fire and Craig Mello



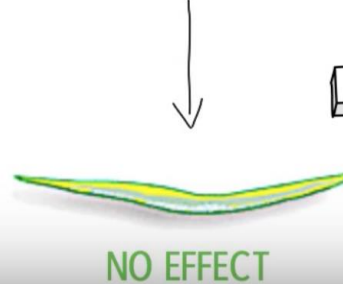
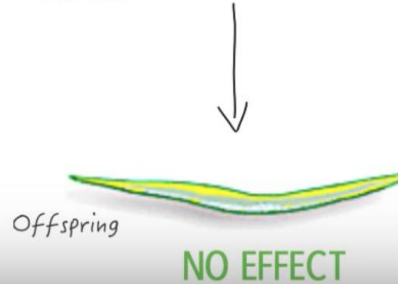
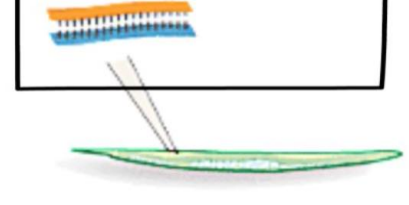
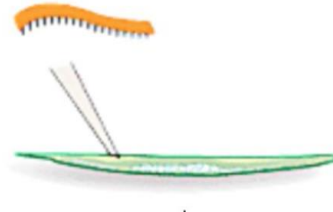
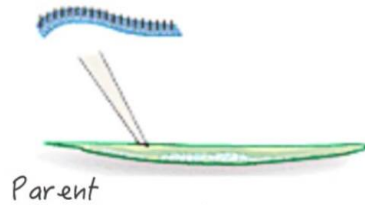
CAENORHABDITIS ELEGANS



Sense RNA

Antisense RNA

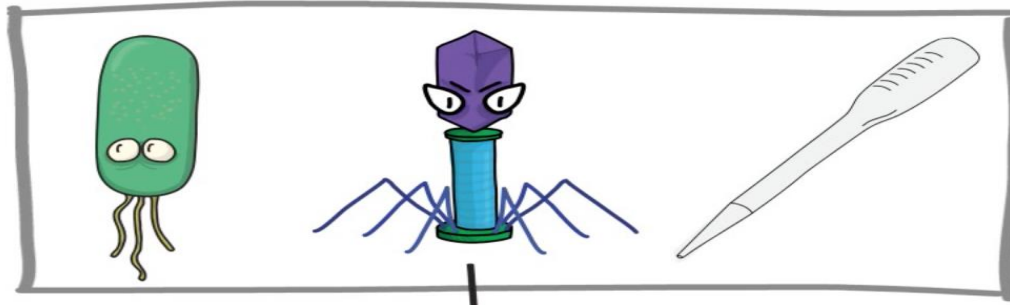
Double-stranded RNA



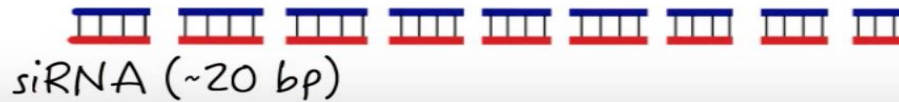
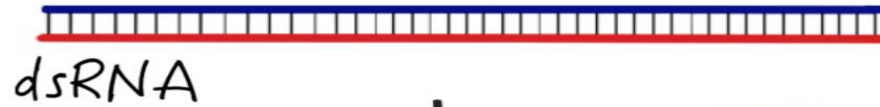
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EXOGENOUS (outside origin)



ENDOGENOUS (inside origin)



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dsRNA



DICER



siRNA (~20 bp)



RISC

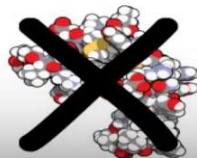
RNA-induced silencing complex

antisense strand of siRNA

mRNA target sequence



mRNA target sequence (cut)



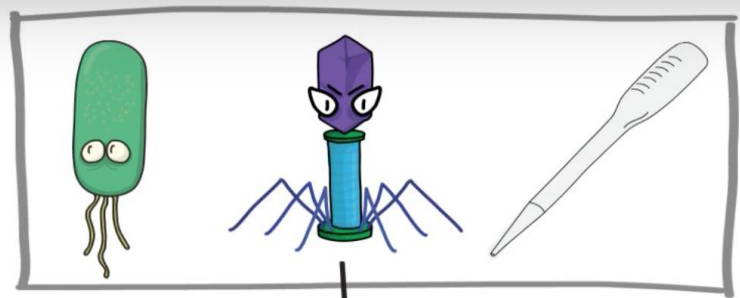
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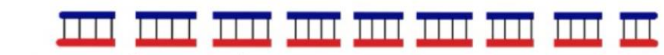


EXOGENOUS (outside origin)

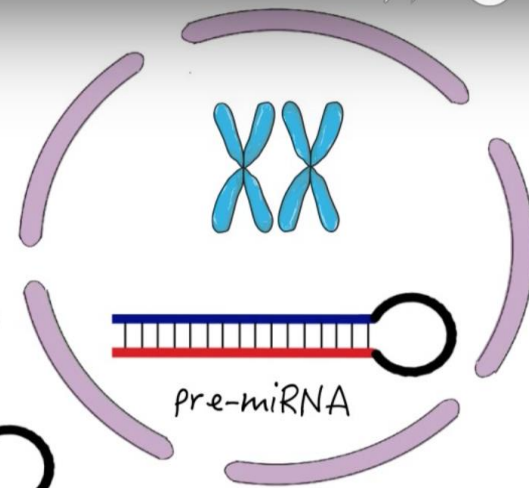
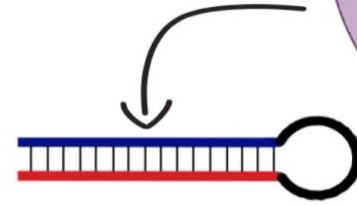
ENDOGENOUS
(inside origin)



dsRNA



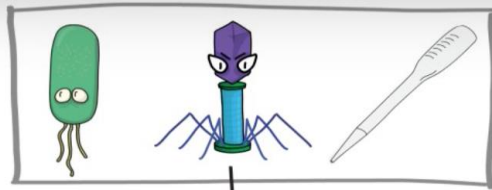
siRNA (~20 bp)



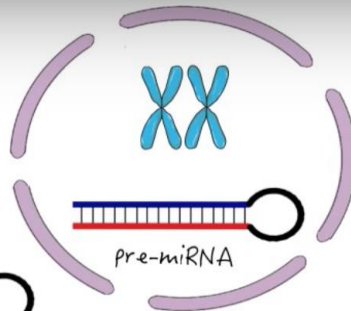
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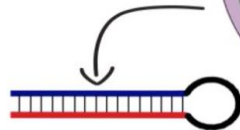
EXOGENOUS (outside origin)



ENDOGENOUS
(inside origin)



dsRNA



DICER

DICER

siRNA (~20 bp)

miRNA

RISC

RNA-induced silencing complex

antisense strand of siRNA

mRNA target sequence

mRNA target sequence (cut)



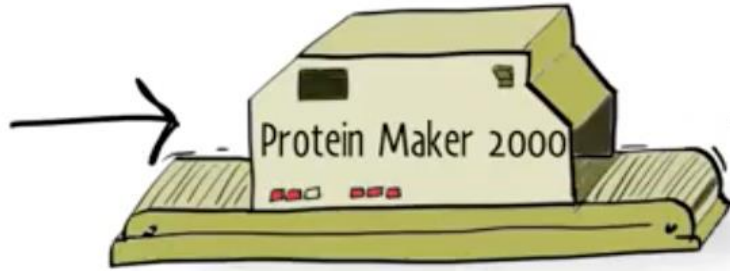
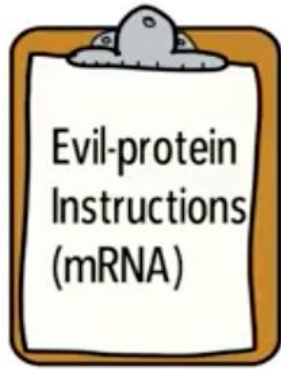
GENE KNOCK-DOWN OR GENE SILENCING



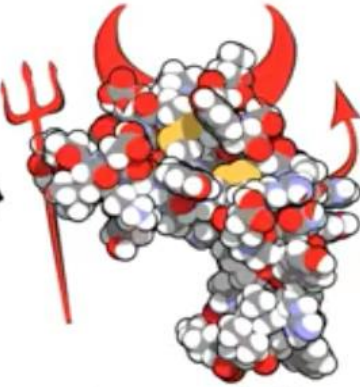
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SO WHAT???



Ribosome



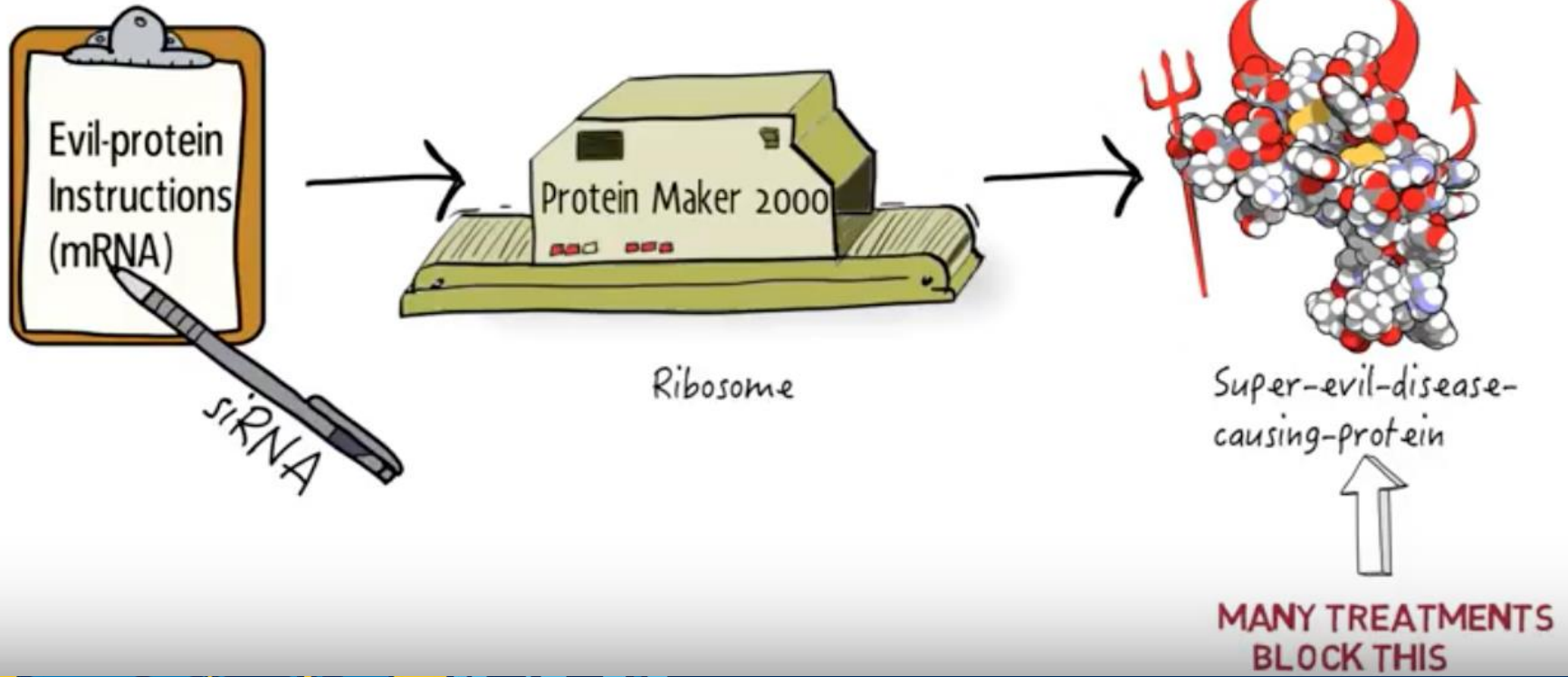
Super-evil-disease-causing-protein



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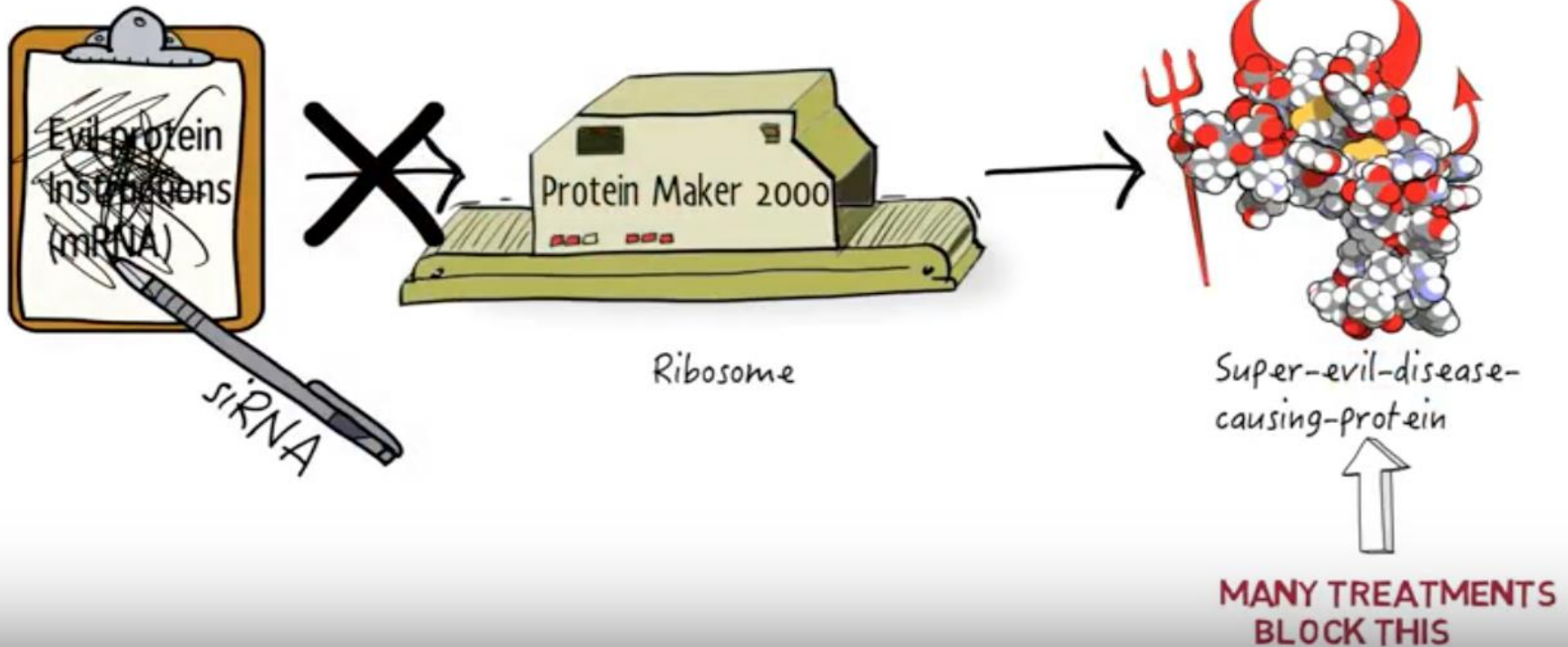
SO WHAT???



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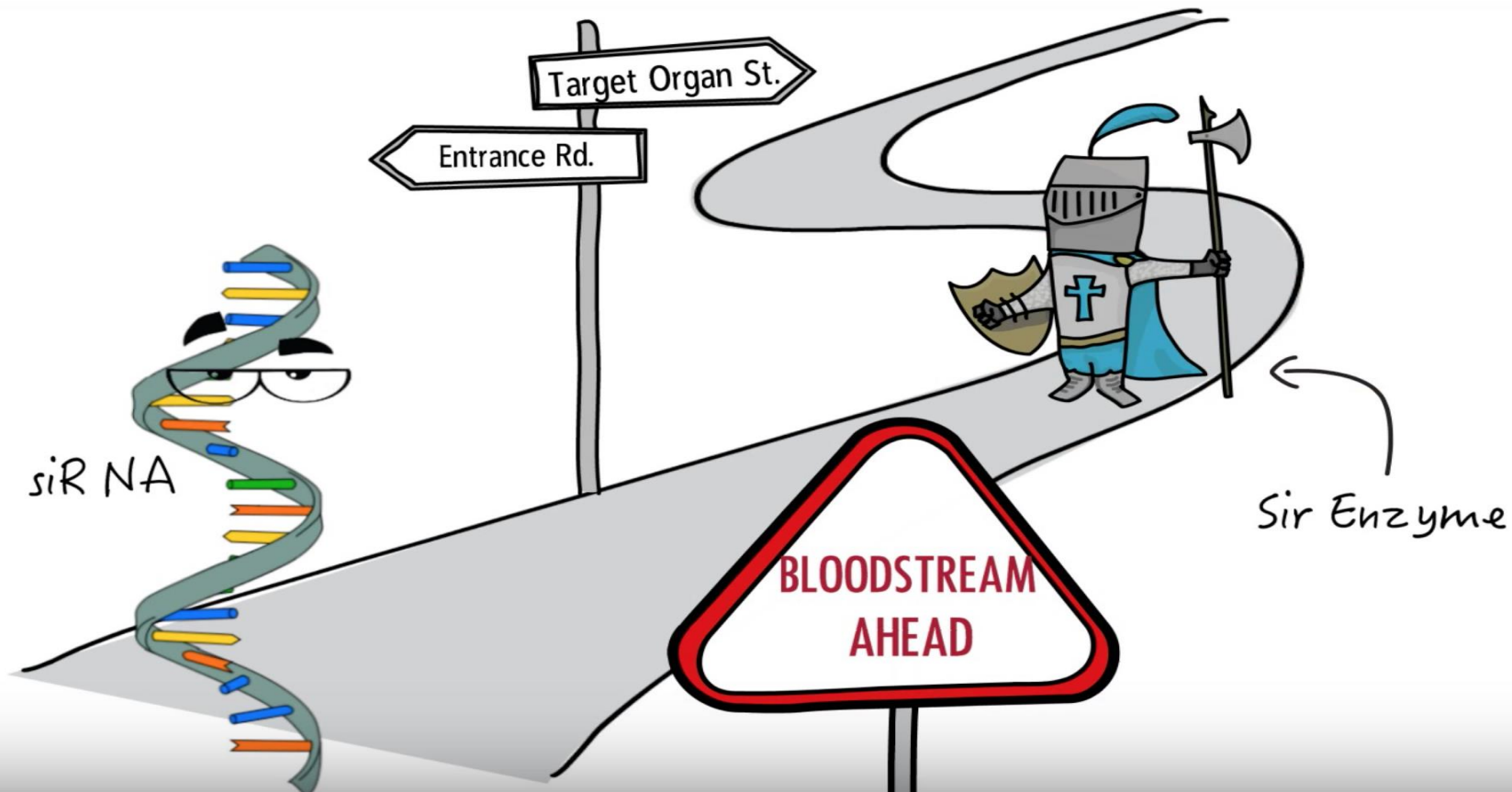


SO WHAT???



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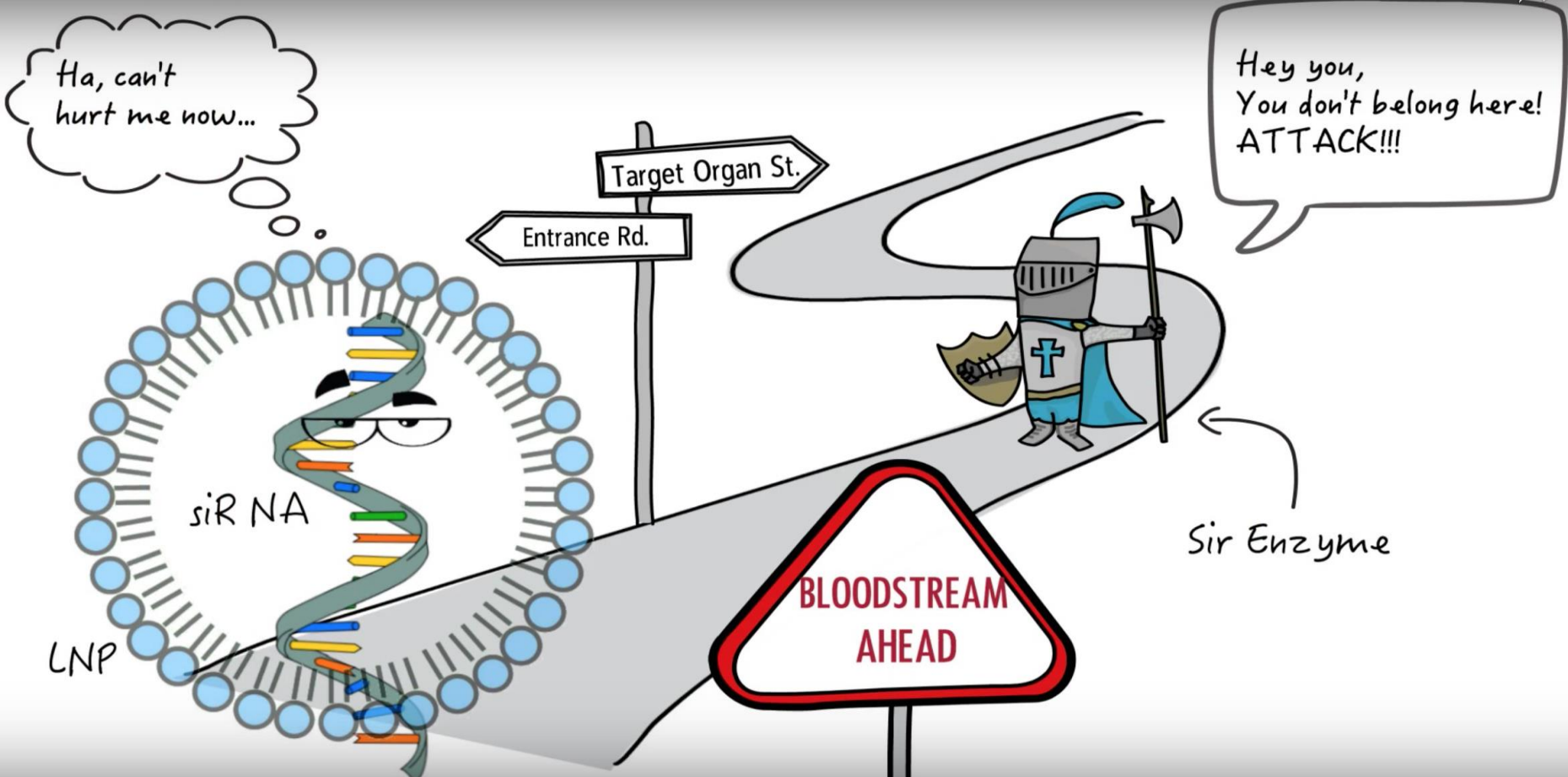




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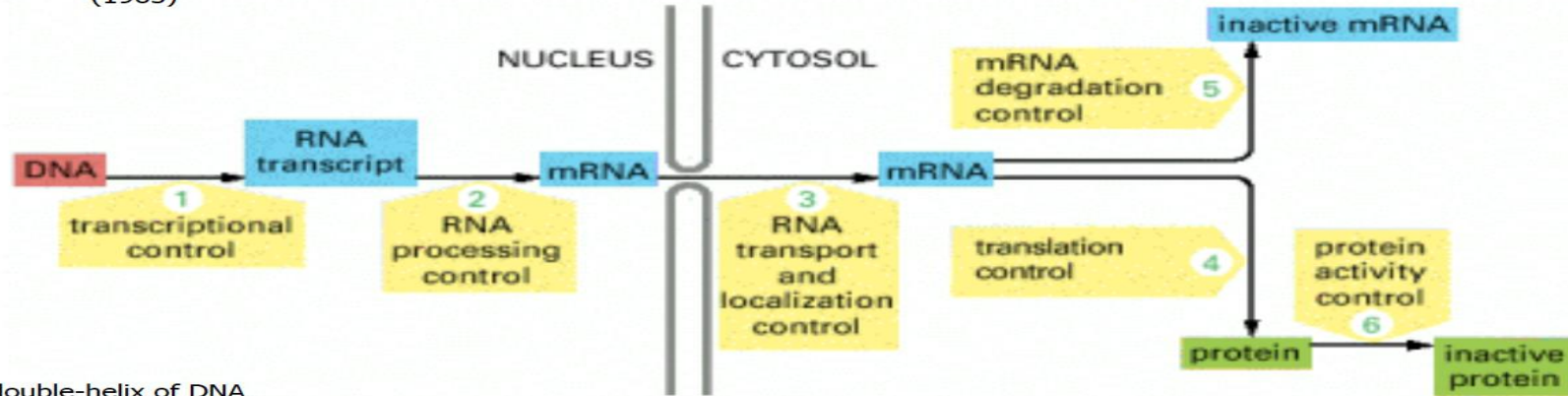
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RNA in the CONTROL of GENE EXPRESSION - a Nobel story

The gene is transcribed into a specific RNA species, mRNA
F. Jacob and J. Monod; Nobel Prize in Physiology or Medicine (1965)

The genetic code
M. Nirenberg and G. Khorana; Nobel Prize in Physiology or Medicine (1968)



The double-helix of DNA identified as the genetic material
F. Crick, J. Watson and M. Wilkins; Nobel Prize in Physiology or Medicine (1962)

The split gene
P. Sharp and R. Roberts; Nobel Prize (1993)

RNA can act as a catalyst
S. Altman and T. Cech; Nobel Prize in Chemistry (1989)



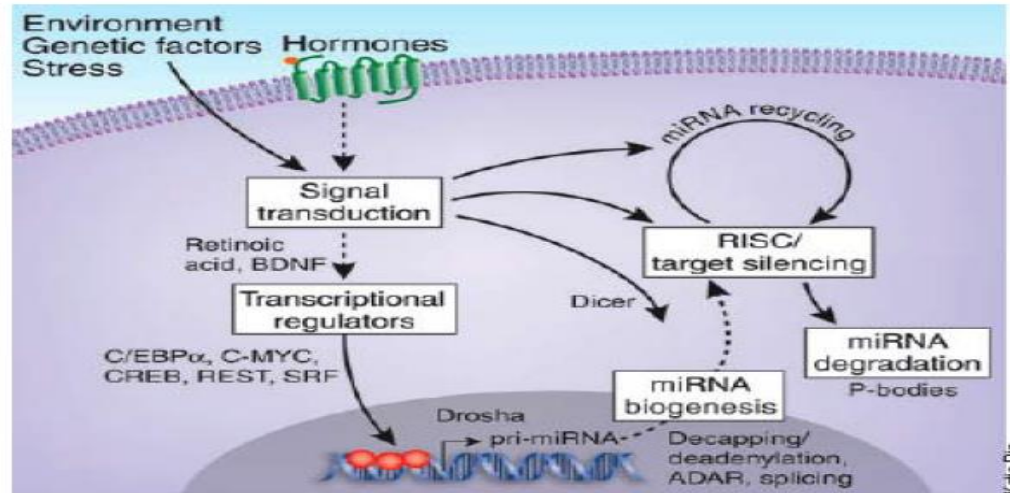
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miRNA FUNCTIONS IN MAMMALS

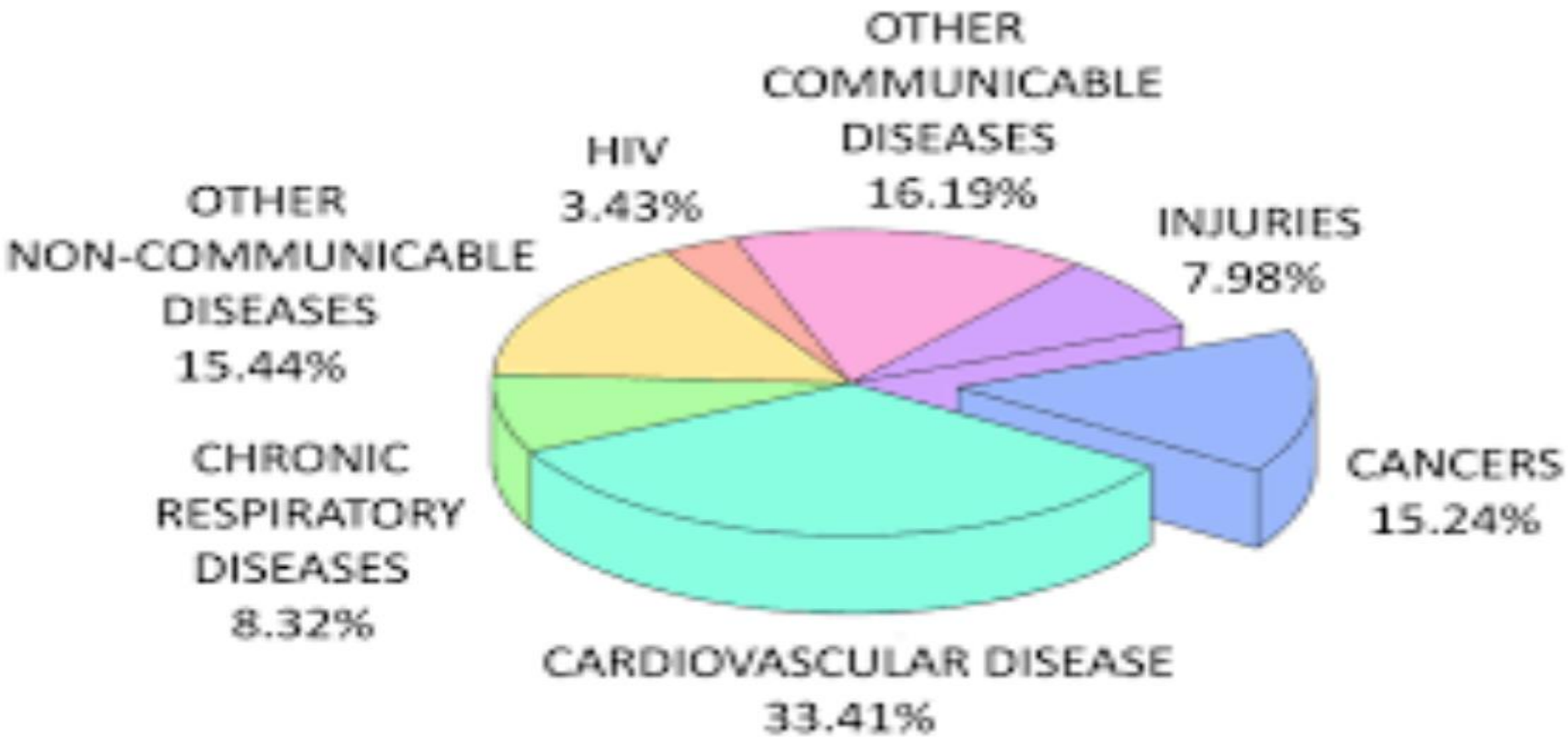
Up to now about 800 miRNA have been discovered in humans, and probably a vast % of human genes are controlled by miRNA activity

- Cell proliferation
- Cell death
- Cell differentiation
- Embryonic development
- Cancer
- Viral infections



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miRNAs are involved in all aspects of cardiovascular function

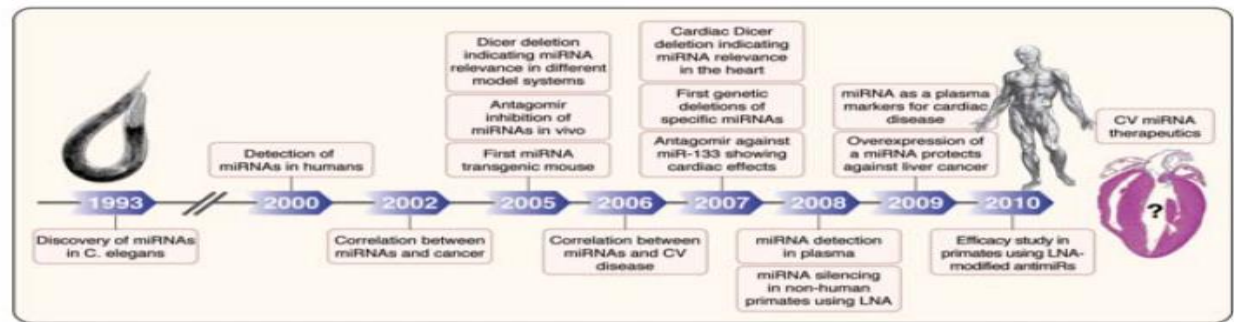


Figure 1. Breakthrough discoveries in miRNA biology. Time line indicating seminal discoveries in miRNA biology with a special focus on the cardiovascular field.

Circ Res. 2011;108:219-234

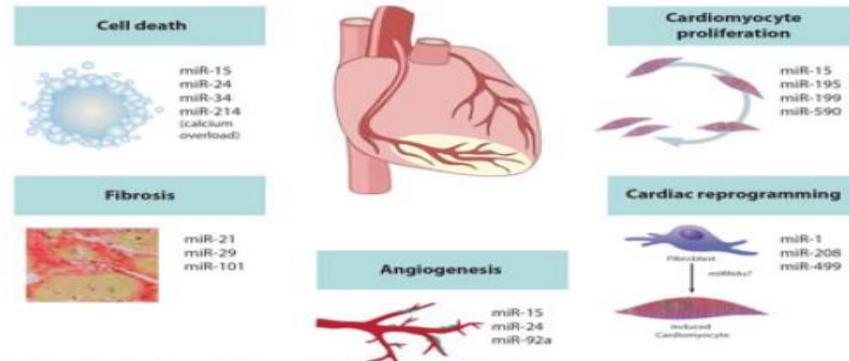


Figure 2. MicroRNAs (MiRNAs) in postinfarction repair.

Arterioscler Thromb Vasc Biol. 2013;33:1739-1746

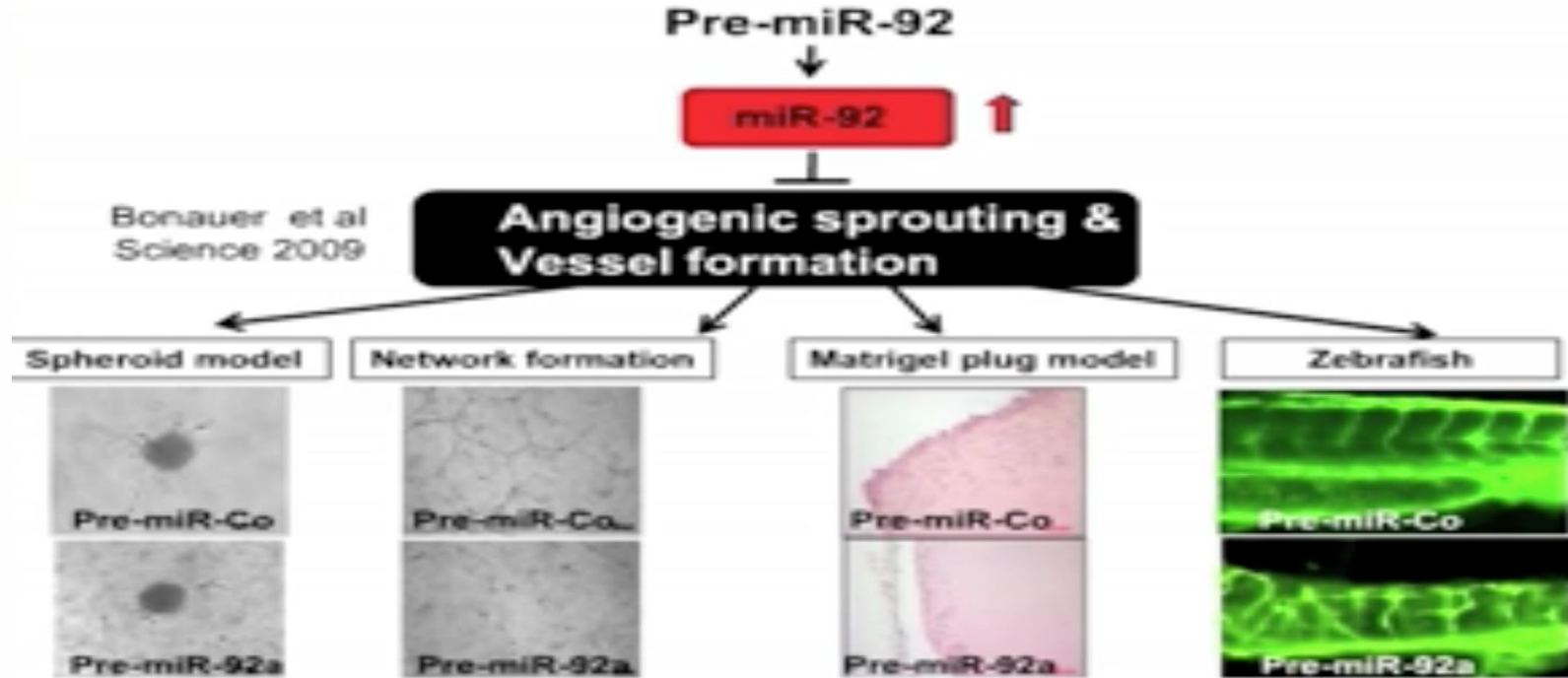
Giacca, M, et al. 2015. *J Mol Cell Cardiol* 89, 68
 Giacca, M 2015. *Mol Ther* 23, 984
 Aguirre, A, et al. 2014. *Cell Stem Cell* 15, 589
 Zacchigna, S, et al. 2014. *Circ Res* 114, 1827



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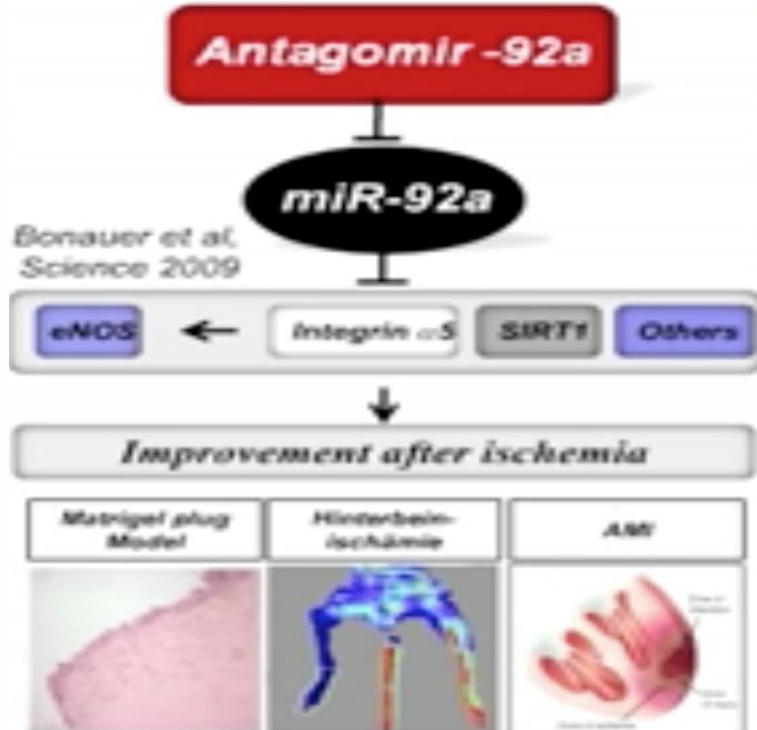
miR-92a regulates angiogenesis



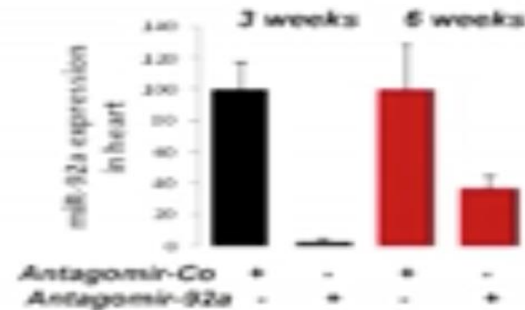
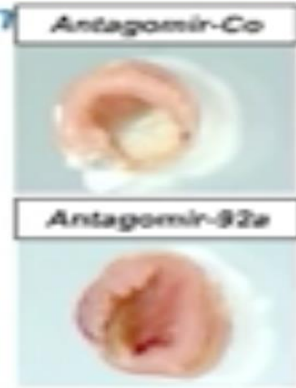
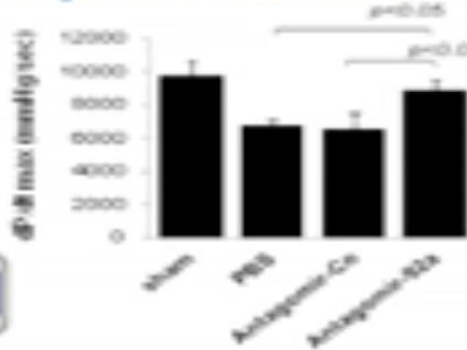
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Antagomir-92 improves the recovery after ischemia



Myocardial infarction



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Inhibition of MicroRNA-92a Protects Against Ischemia/Reperfusion Injury in a Large-Animal Model

Animals: male pigs, German landrace, body weight: 20-25kg

- 1) Induction of acute myocardial infarction (AMI) by occlusion of the left anterior descending (LAD) using a PTCA balloon
- 2) After 55 minutes of ischemia: Infusion of LNA-92a (5 mg/kg heart weight; 0.03mg/kg body weight; 0.75mg/pig)
- 3) Reperfusion after 60 minutes of ischemia
- 4) Harvest 72 hours after AMI



Endpoints:

- Infarct size
- Global myocardial function
- Regional myocardial function
- Inflammation
- Cell death
- Collection of tissue samples for qPCR, Western blot and Histology



Rabea Hinkel & Christian Kupatt, Munich



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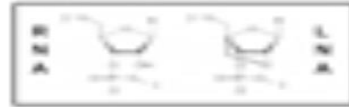


Inhibition of MicroRNA-92a in pigs after Ischemia/Reperfusion Injury

LNA-92a

5'-TACGGTGGGAGACAGAGGAGCAAT-3'

● DNA
● LNA



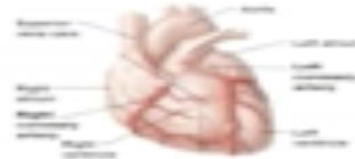
Diagrams showing heart weight

Hinkel et al,
Circulation 2013

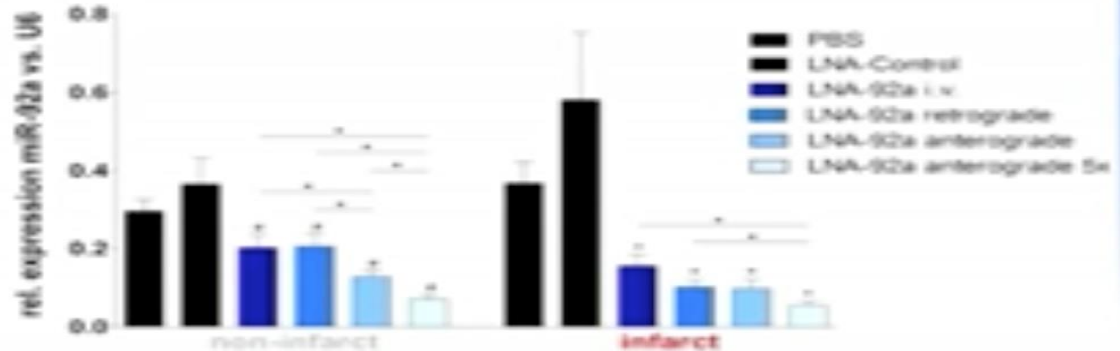
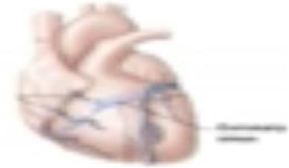
Delivery:

- i.v.
- Anterograde
- Retrograde

anterograde



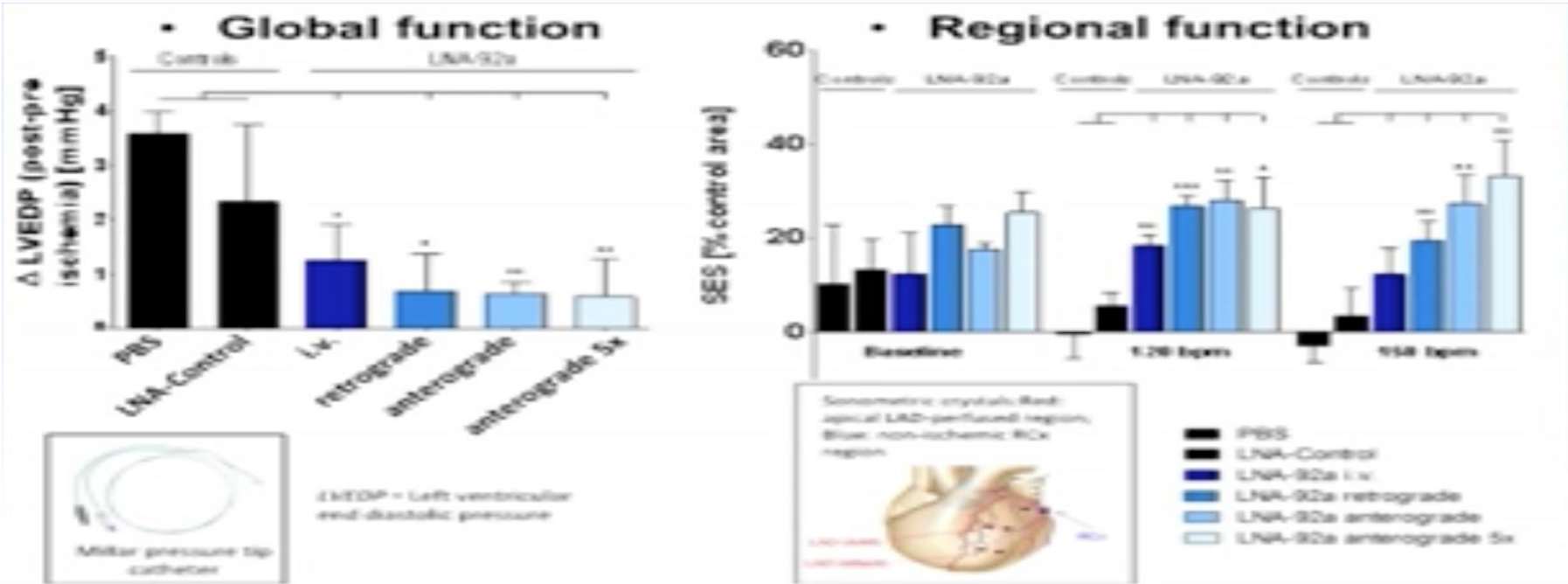
retrograde



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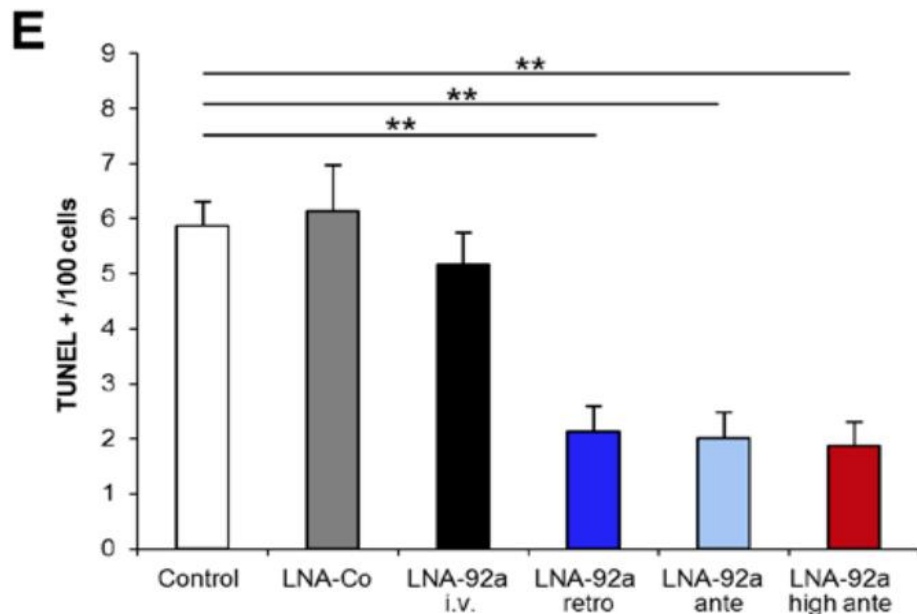
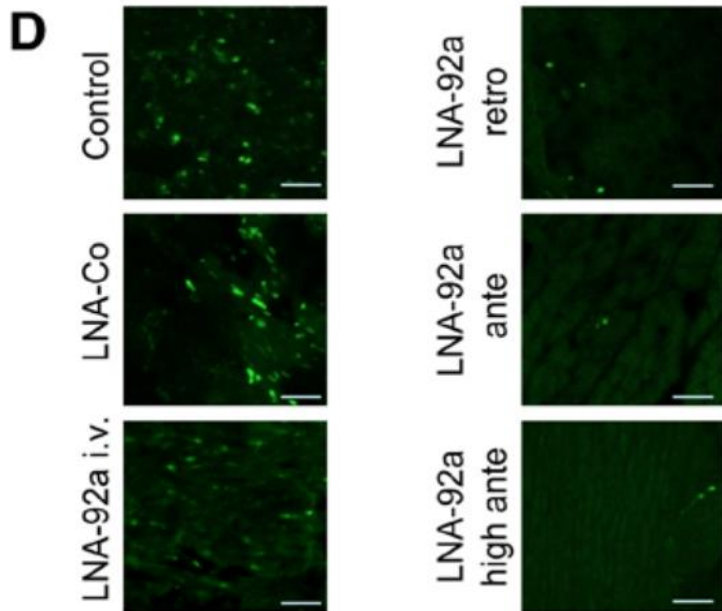
LNA-92a Improve cardiac function



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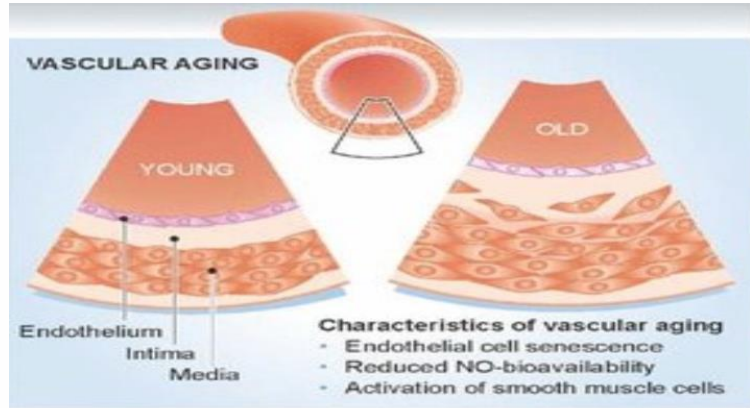
LNA-92a increases capillary density



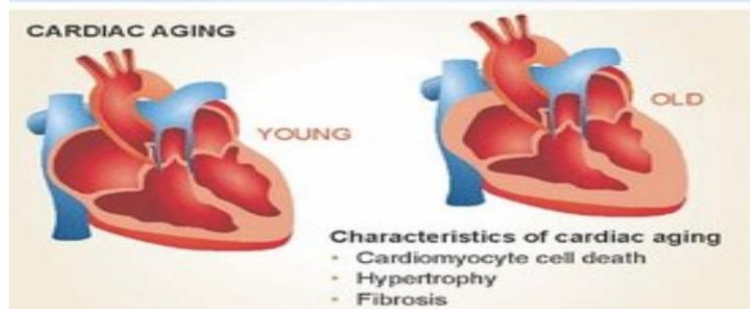
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Regulation of miRNAs during cardiovascular aging



miRNA	Targets	Functions
miR-29 ↑	Matrix proteins • Collagens • Elastin	• miR-29 inhibition reduces aneurysm formation
miR-34 ↑	• SIRT1 • Bcl2 • Cdk4 • Cyclin D2	• Induces endothelial and pro-angiogenic cell senescence • Induces cell death of pro-angiogenic cells
miR-217 ↑	• SIRT1	• Induces endothelial cell senescence and reduces NO availability
miR-146 ↑	• IRAK	• Increased in cultured endothelial cells and linked to inflammatory response
miR-146 ↓	• NOX4	• Reduced in cultured endothelial cells and linked to generation of reactive oxygen species



miR-22 ↓	• Mimecan/ osteoglycine • SIRT1	• Induces senescence of cardiac fibroblasts
miR-18 ↓ miR-19 ↓	• Thrombospondin-1 • Connective tissue growth factor	• Suppress collagen expression



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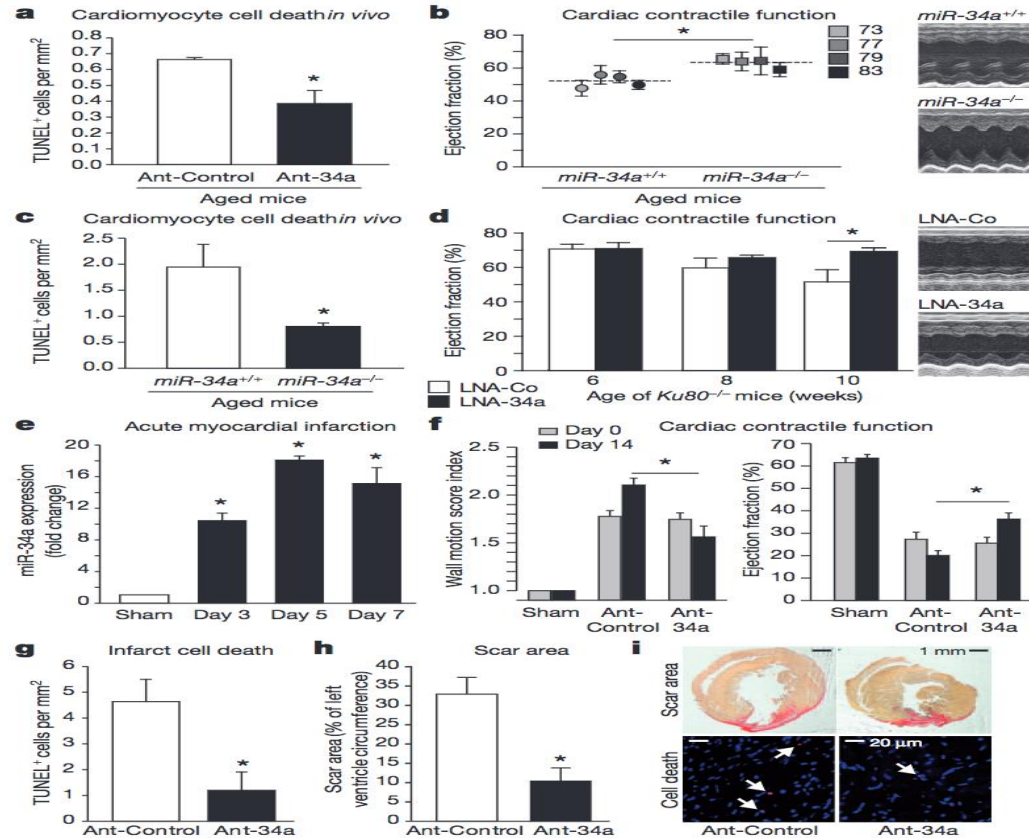


Figure 2 | miR-34a reduction inhibits age-related and myocardial infarction-induced cardiomyocyte cell death and cardiac function.



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Table 1 Common circulating miRNAs in cardiovascular diseases.

Groups	miRNAs	Sources	RNA Isolation	Expression	miRNA Detection	Potential value	References
AMI	miR-208	Rat	Plasma	Up-regulation	Microarray and qRT-PCR	Diagnostic biomarker	[23]
	miR-208a	Rat, Human	Plasma	Up-regulation	Microarray and qRT-PCR	Diagnostic biomarker	[6]
	miR-208b	Human	Plasma	Up-regulation	qRT-PCR	Diagnostic biomarker	[24,30]
	miR-499	Human	Plasma	Up-regulation	qRT-PCR	Diagnostic biomarker	[24,25,30]
	miR-1	Human	Plasma	Up-regulation	qRT-PCR	Diagnostic biomarker	[30,63]
	miR-133	Human	Plasma	Up-regulation	qRT-PCR	Diagnostic biomarker	[60]
	miR-30a	Human	Plasma	Up-regulation	qRT-PCR	Diagnostic biomarker	[61]
	miR-150	Human	Plasma	Down-regulation	qRT-PCR	Prognostic biomarker	[62]
	let-7b	Human	Plasma	Down-regulation	qRT-PCR	Diagnostic biomarker	[61]
	miR-126	Human	Plasma	Down-regulation	qRT-PCR	Diagnostic biomarker	[63]
	miR-423	Human	Plasma	Up-regulation	Microarray and qRT-PCR	Diagnostic biomarker	[9]
	miR-18b						
	miR-499	Human	Plasma	Up-regulation	qRT-PCR	Diagnostic biomarker	[24]
	miR-103, miR-142	Human	Plasma	Down-regulation	Microarray	Diagnostic biomarker	[42]
HF	miR-320a, miR-22	Human	Serum	Up-regulation	qRT-PCR	Prognostic biomarker	[43]
	miR-16, miR-20b, miR-26b	Rat	Plasma	Up-regulation	Microarray and qRT-PCR	Disease progression and therapeutic efficacy	[45]
		Human	Plasma, Serum	Up-regulation	qRT-PCR	Diagnostic and prognostic biomarker	[47]
	miR-296, miR-133b, miR-625, miR-1236	Human	Plasma	Down-regulation	Microarray and qRT-PCR	Pathogenesis of essential hypertension	[10]
	let-7e, Hcmv-miR-UL112, miR-605, miR-623, miR-516b	Human	Plasma	Up-regulation	Microarray and qRT-PCR	Pathogenesis of essential hypertension	[10]
	miR-132, miR-212	Human, Rat	Plasma	Up-regulation	Microarray and qRT-PCR	Mechanisms of essential hypertension	[51]
	miR-145	Human	Blood	Up-regulation	qRT-PCR	Diagnostic biomarker	[53]
	miR-21	Human	Plasma	Up-regulation	qRT-PCR	Diagnostic biomarker	[54]
	miR-221	Human	Plasma	Down-regulation	qRT-PCR	Diagnostic biomarker	[54]
	miR-210	Human	Blood	Down-regulation	qRT-PCR	Diagnostic and prognostic biomarker	[55]
Stroke	miR-290	Rat	Blood	Up-regulation	Microarray and qRT-PCR	Diagnostic biomarker and mechanisms of stroke	[56]
	let-7i	Rat	Blood	Down-regulation	Microarray and qRT-PCR	Diagnostic biomarker and mechanisms of stroke	[56]
	miR-298	Rat	Blood	Up-regulation	Microarray	Diagnostic biomarker	[57]
	miR-155	Rat	Blood	Down-regulation	Microarray	Diagnostic biomarker	[57]
	miR-30a, miR-126	Human	Plasma	Down-regulation	qRT-PCR	Diagnostic biomarker	[60]

Note: AMI: Acute myocardial infarction, HTN: Hypertension, HF: Heart failure, qRT-PCR: quantitative reverse transcription-polymerase chain reaction.

Common circulating miRNAs in cardiovascular disease

Heart, Lung and Circulation (2014) 23, 503–510

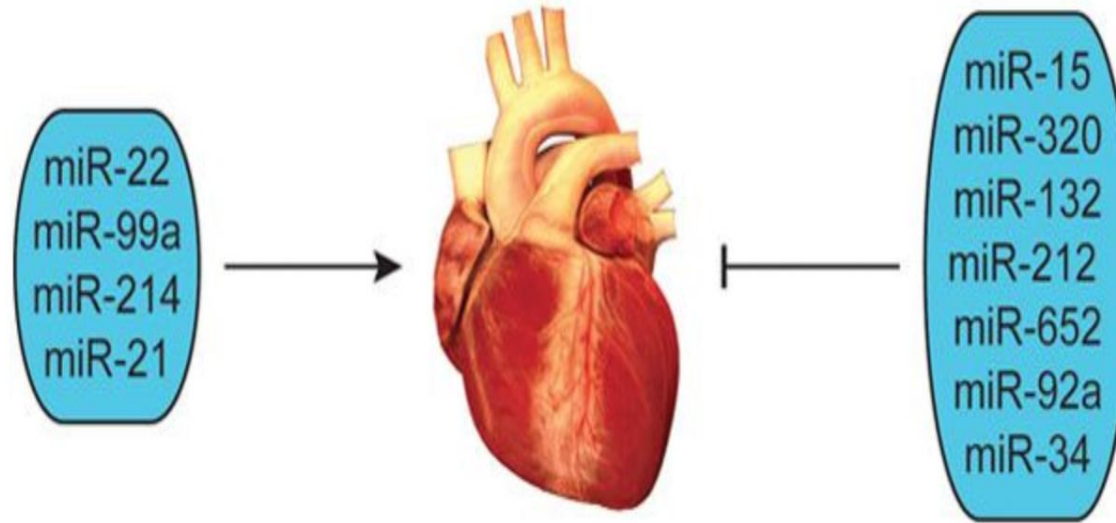


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From: miRNAs in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges



miRNAs as therapeutic targets for cardiovascular diseases. Increased expression (arrow head) or decreased expression (bar-head) provides beneficial or protective effects in the treatment of CVD.



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Therapeutic Manipulation of miRNAs

miRNAs in Heart Failure: miR-133 is highly abundant in cardiomyocytes, but is reduced in animal models of hypertrophy and in patients with hypertrophic cardiomyopathy

miRNAs in Neointima Formation and Atherosclerosis: In addition to its profibrotic role, miR-21 enhances neointimal growth through pro-proliferative and antiapoptotic effects on vascular smooth muscle cells (SMCs). Inhibition of miR-21 reduces in-stent restenosis in animals

Barwari et al. JACC VOL. 68, NO. 23, 2016



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miRNAs in Lipid Metabolism: Systemic inhibition of miR-148a caused a significant reduction in plasma low-density lipoprotein cholesterol, but also increased high-density lipoprotein cholesterol levels. Long-term side effects of miR-122 and miR-33 inhibition, combined with the advent of novel therapeutic options for [dyslipidemia](#), may limit the clinical use of miRNAs to modulate [lipid metabolism](#).

Barwari et al. JACC VOL. 68, NO. 23, 2016



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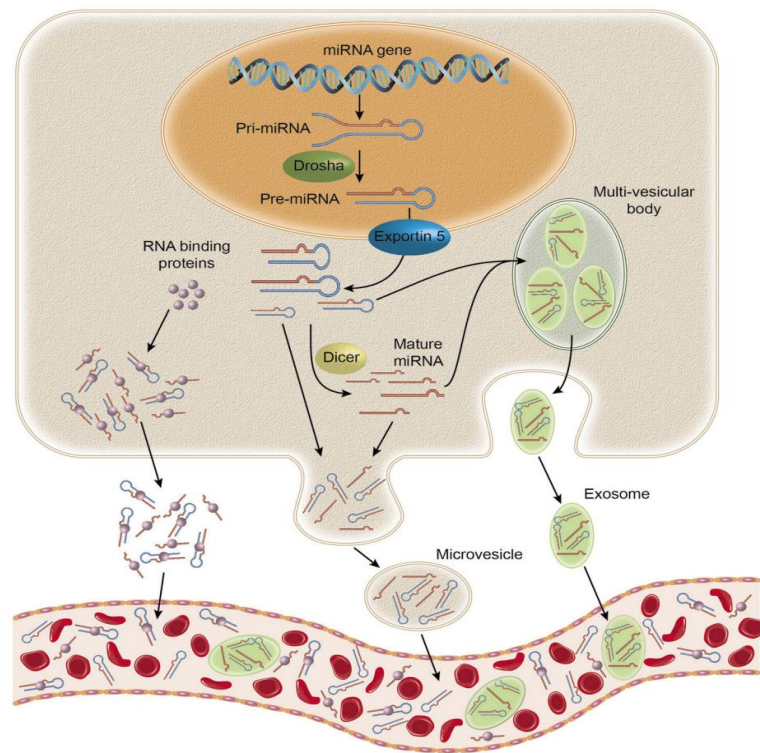


Figure 1. Biogenesis of circulating miRNAs. miRNAs are transcribed in the nucleus as pri-miRNAs with 5'-caps and 3'-polyA tails. Drosha removes the cap and polyA tail to generate pre- miRNAs, which are exported from the nucleus via Exportin 5. In the cytoplasm Dicer processes the pre-miRNA to mature miRNAs. Pre-miRNA and mature miRNA can (a) bind to RNA-binding proteins to be directly excreted from the cell, (b) packaged into microvesicles or (c) packed into exosomes and multi-vesicular bodies where the exosomes are then released. Pre-miRNA and mature miRNAs are taken into the bloodstream by endocytosis, binding to receptors or via membrane fusion.

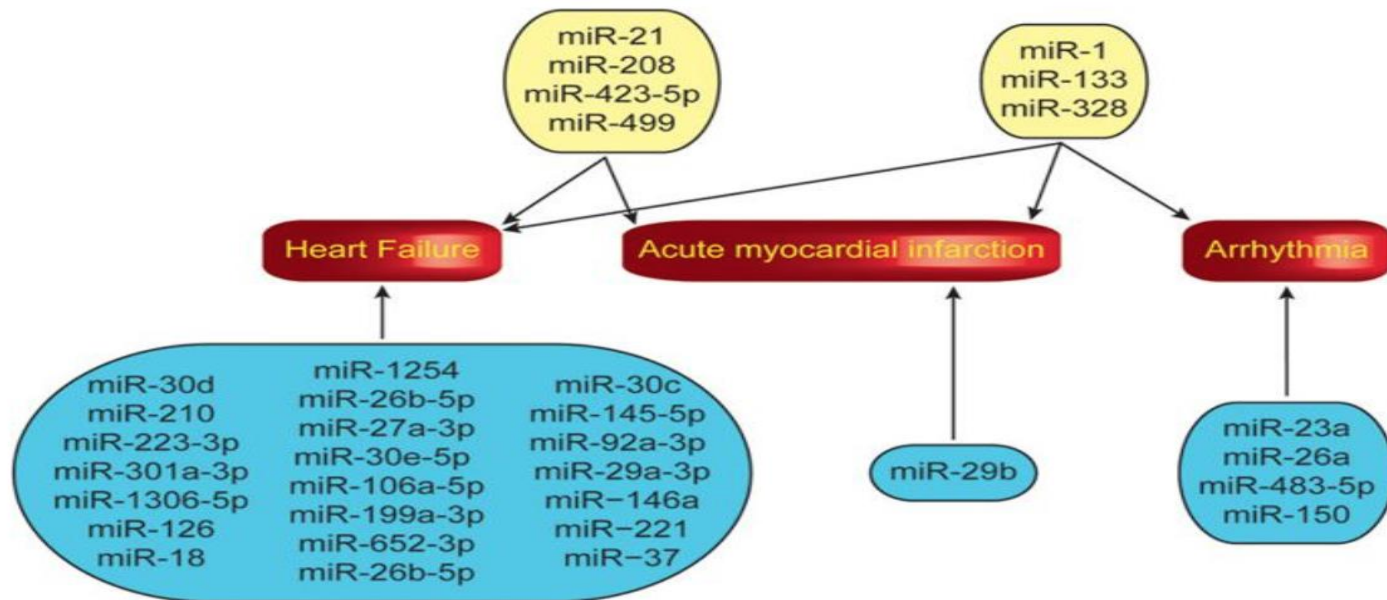


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miRNAs in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges



miRNAs associated with the diagnosis and prognosis of heart failure, acute myocardial infarction and arrhythmia. miRNAs in blue boxes are associated with a single pathology, while those in yellow boxes with multiple pathologies.



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Conclusion

1. Caution needs to be exercised to avoid moving toward clinical evaluation too quickly..
2. Targeting individual miRNAs therefore requires meticulous evaluation of systemic effects
3. A careful approach in advancing miRNA therapies may slow progression toward clinical application, but may spare miRNA therapeutics a setback similar to [gene therapy](#).
4. The great potential of miRNAs justifies the exercise of apprehension before large-scale clinical studies for CVD.



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