The Science of RNA interference (RNAi)

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The Science of RNA interference (RNAi)

- This resource provides information about RNAi.
- This resource is intended to be viewed in its entirety for educational and scientific exchange purposes and is not intended as recommendations for clinical practice.
- This resource may contain hyperlinks that are not functional in this format.
- For further information, please see RNAiScience.com to connect with a Medical Science Liaison, submit a medical information request, or access other Alnylam medical and scientific information resources.

Contents

RNA, ribonucleic acid; RNAi, RNA interference; siRNA, small interfering RNA.

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Natural RNAi Mechanism

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RNA Interference (RNAi) Is a Naturally Occurring Mechanism for Silencing Gene Expression[1–3]

RNAi uses siRNA or miRNA to knock down expression of target genes by distinctive mechanisms[1–4]

What are siRNA and miRNA?

siRNA and miRNA are types of short non-coding RNAs that target mRNA to silence genes[4]

What is the difference between siRNA and miRNA?

siRNA and miRNA have similar structures but slightly different mechanisms of action[3,4]

The main difference between siRNA and miRNA is that siRNA inhibits the expression of one specific target mRNA, while miRNA can regulate expression of multiple mRNAs[4]

How do siRNA and miRNA silence gene expression?

siRNAs and miRNAs both use endogenous RNA-induced silencing complex (RISC) to induce silencing of gene expression[1,3,4]

What is RISC and how does it work?

RISC is a ribonucleoprotein complex formed when an siRNA or miRNA is loaded onto a member of the Argonaute protein family[5,6]

The bound siRNA or miRNA guides RISC to target complementary mRNAs[3–5]

RISC bound to miRNA silences gene expression mainly through translational repression or degradation of the target mRNA. RISC bound to siRNA silences gene expression at the post-transcriptional level through cleavage of the target mRNA[3–5]

This material will focus on the siRNA pathway

mRNA, messenger RNA; miRNA, microRNA; RNA, ribonucleic acid; siRNA, small interfering RNA.

1. Niemietz et al. *Molecules* 2015;20:17944–75; 2. Hu et al. *Signal Transduct Target Ther* 2020;5:101; 3. Chery. *Postdoc J* 2016;4:35–50; 4. Lam et al. *Mol Ther Nucleic Acids* 2015;4:e252; 5. Iwakawa & Tomari. *Molecular Cell* 2022;82:30–43; 6. Jadhav et al. *Nat Biotechnol* 2024;42:394–405.

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The Natural RNAi Mechanism of Action Involves Key Elements Such as Dicer and RISC[1–4]

RNAi using siRNA[1–4]

1. Long dsRNA is processed into shorter strands in the nucleus by Drosha, an RNase enzyme
2. Shorter dsRNA is exported to the cytoplasm, and cleaved into siRNA by Dicer, another RNase enzyme
3. siRNA is loaded onto the multiprotein structure known as RISC and unwinds into passenger and guide strands
4. The passenger strand is degraded in the cytoplasm, and the RISC + guide strand bind to complementary target mRNA
5. Target mRNA is cleaved at a specific site and then degraded, decreasing production of the target protein

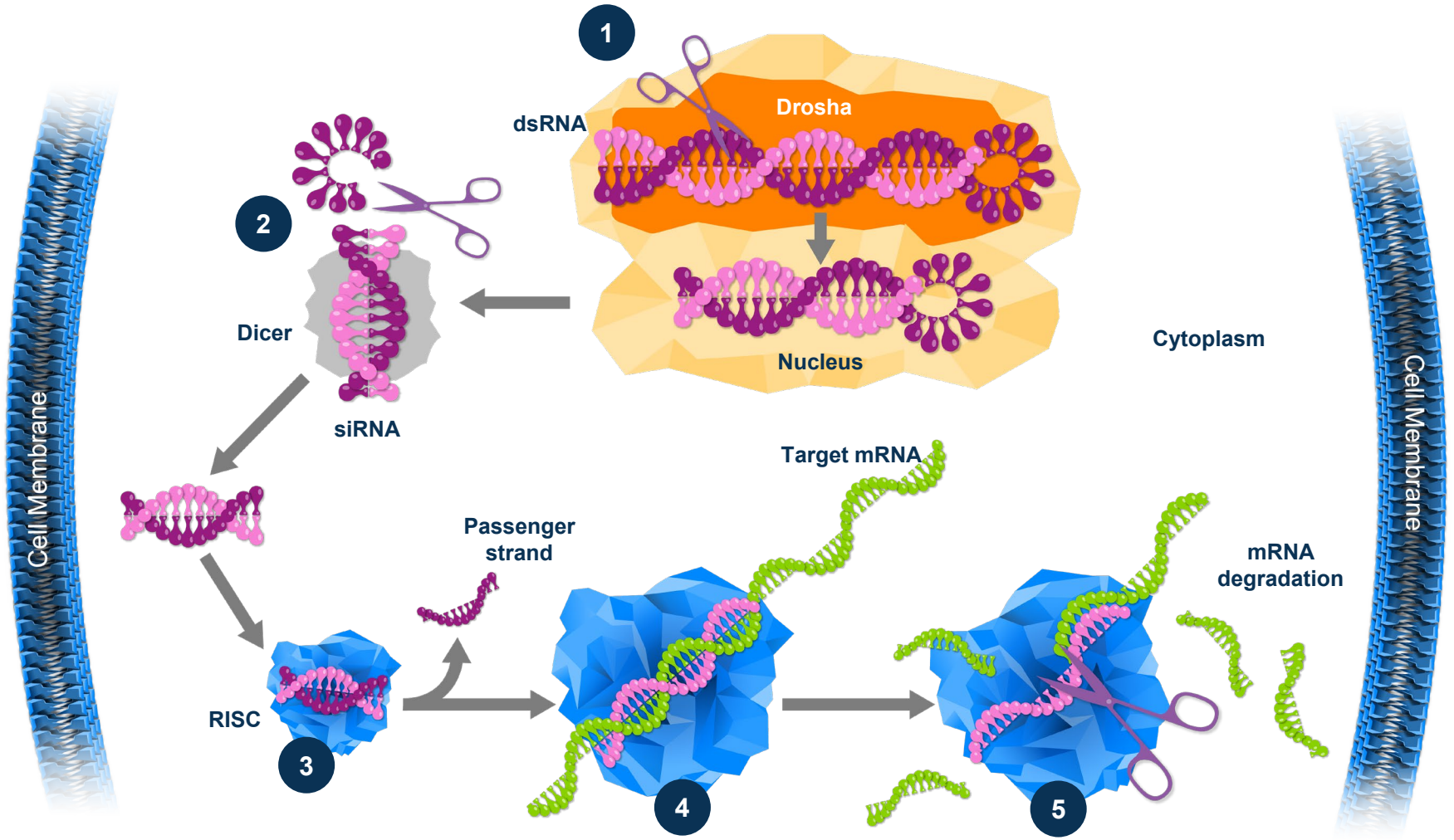

Figure adapted from UMass Chan Medical School RNA Therapeutics Institute. Original figure created by Angela Messmer-Blust, RNA Therapeutics Institute, UMass Chan, using BioRender[4]

ds, double-stranded; mRNA, messenger RNA; RISC, RNA-induced silencing complex; RNA, ribonucleic acid; RNAi, RNA interference; RNase, ribonuclease; siRNA, small interfering RNA.

1. Niemietz et al. *Molecules* 2015;20:17944–75; 2. Hu et al. *Signal Transduct Target Ther* 2020;5:101; 3. Chery. *Postdoc J* 2016;4:35–50; 4. UMass Chan Medical School RNA Therapeutics Institute. Available from: https://www.umassmed.edu/rti/biology/rna/how-rnai-works/ (accessed July 15, 2024).

RNAi Therapeutics

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RNAi Therapeutics Can Be Synthesized to Target Disease-causing Genes[1,2]

RNAi therapeutics alter gene expression without editing the target gene itself[3]

RNAi therapeutics are designed with a minimal number of phosphorothioate modifications, which reduces the likelihood of non-specific protein binding that could lead to off-target effects, but still provides protection against nuclease degradation[4–10]

Some RNAi therapeutics are already approved and available to patients, and many more are in late-stage clinical development across a diverse spectrum of diseases[1,2]

- RNAi therapeutics target the underlying mechanism of disease by providing rapid knockdown of a target gene[1,2]

RNAi, ribonucleic acid interference.

1. Jay et al. *Int J Cardiovasc Sci* 2021;35:665–75; 2. Zhang et al. *Biochem Pharmacol* 2021;189:114432; 3. Kim. *Exp Mol Med* 2022;54:455–65; 4. Anderson et al. *Nucleic Acids Res* 2021;49:9026–41; 5. Crooke et al. *J Biol Chem* 2021;296:100416; 6. Shen et al. *Nat Biotechnol* 2019;37:640–50; 7. Flierl et al. *J Exp Med* 2015;212:129–37; 8. Frazier. *Toxicol Pathol* 2015;43:78–89; 9. Roberts et al. *Nat Rev Drug Discov* 2020;18:673–94; 10. Friedrich & Aigner. *BioDrugs* 2022;36:549–71.

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RNAi Therapeutics Leverage the Natural RNAi Mechanism to Decrease Production of the Target Protein[1–4]

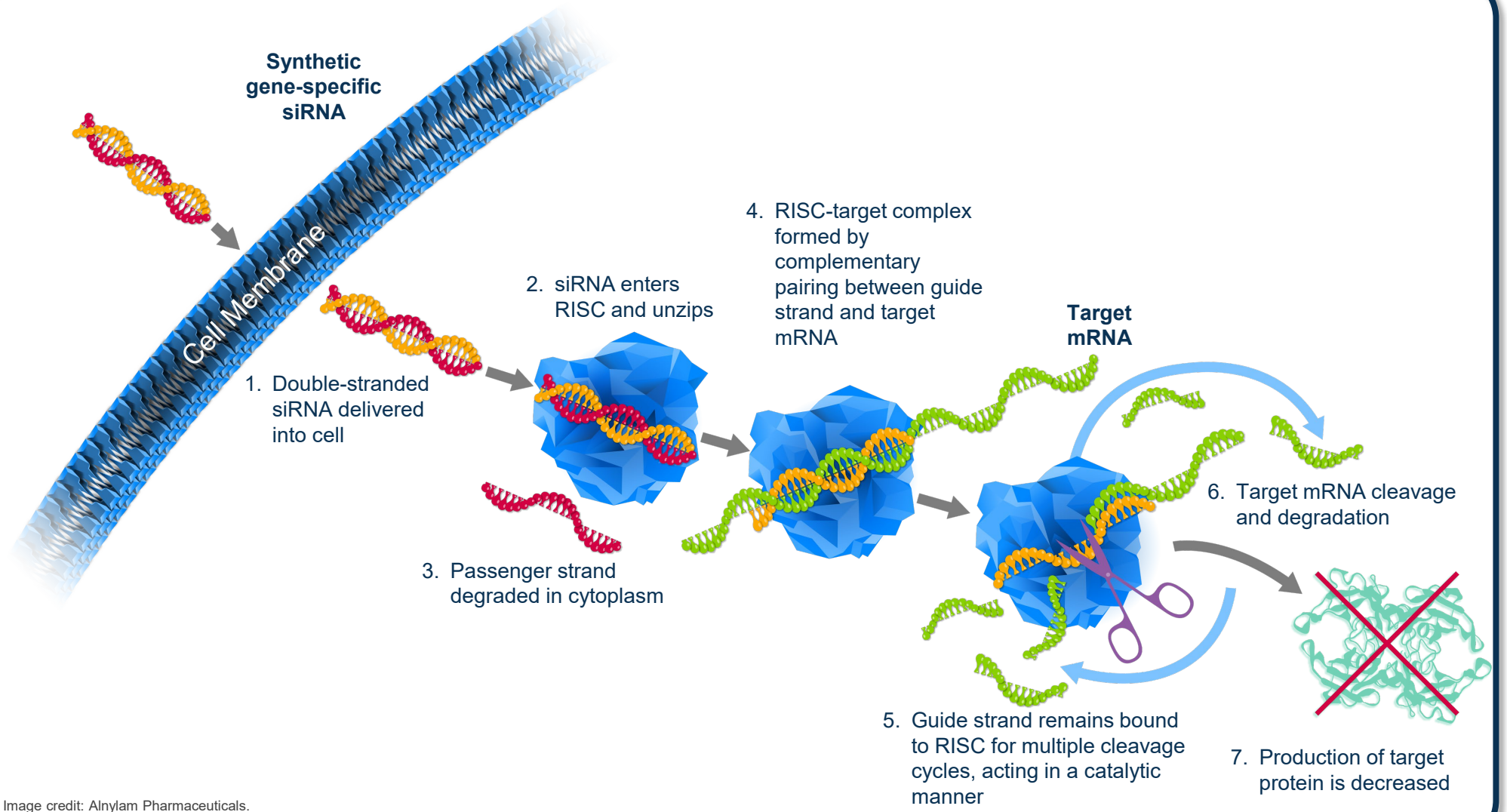

Image credit: Alnylam Pharmaceuticals.
Figure adapted from information in Friedrich & Aigner. *BioDrugs* 2022;36:549–71 and Niemietz et al. *Molecules* 2015;20:17944–75, plus data published in Coelho et al. *N Engl J Med* 2013;369:819–29[1,2,4]

- Based on Nobel Prize-winning scientific discovery[5]
- Leveraging the naturally occurring mechanism for silencing of gene expression[1–3]
- A single siRNA bound to RISC is recycled and can cleave multiple mRNAs during its lifetime,[1–3,6] and can cause a rapid, targeted, and sustained decrease in the levels of disease-causing protein[1–3,7,8]

Scan QR code for video content: *RNAi Therapeutics: How Do They Work?*

mRNA, messenger RNA; RISC, RNA-induced silencing complex; RNA, ribonucleic acid; RNAi, RNA interference; siRNA, small interfering RNA.

1. Friedrich & Aigner. *BioDrugs* 2022;36:549–71; 2. Niemietz et al. *Molecules* 2015;20:17944–75; 3. Jay et al. *Int J Cardiovasc Sci* 2021;35:665–7; 4. Coelho et al. *N Engl J Med* 2013;369:819–29; 5. Montgomery. *Nat Struct Mol Biol* 2006;13:1039–41; 6. Hutvagner & Zamore. *Science* 2002;297:2056–60; 7. Raal et al. *N Engl J Med* 2020;382:1520–30; 8. Keam. *Drugs* 2022;82:1419–25.

II Delivering siRNAs to the Liver

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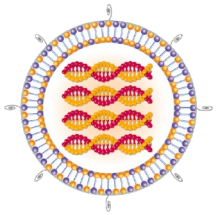
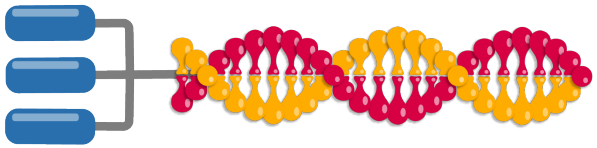
Alnylam Has Developed Two Clinically Validated Modalities for Targeted siRNA Delivery to the Liver, Where Disease-causing Proteins May Be Synthesized[1–4]

	Lipid nanoparticles (LNPs)	GalNAc–siRNA conjugates
siRNA delivery platform		
Delivery mechanism	Synthetic siRNAs encapsulated in LNPs[1,2]	Metabolically stabilized synthetic siRNA conjugated to a GalNAc ligand[2,3]
Structure and size	Multi-component particle system (four different lipids plus the siRNA) of <100 nm diameter[1]	One tris-GalNAc molecule conjugated to the sense strand of a ds-siRNA molecule[2–4]
Delivery to liver	Natural pathway involving association with targeting ligands (e.g. ApoE) of receptors expressed on the surface of hepatocytes[1]	Natural pathway involving the GalNAc ligand binding to the ASGPR on hepatocytes[2,3]
Administration method	IV infusion[1,2]	SC injection[2,3]
Example	Patisiran[2]	Vutrisiran, givosiran, lumasiran[3,4]

ApoE, apolipoprotein E; ASGPR, asialoglycoprotein receptor; ds, double-stranded; GalNAc, N-acetylgalactosamine; IV, intravenous; SC, subcutaneous; siRNA, small interfering ribonucleic acid.

1. Tam et al. *Pharmaceutics* 2013;5:498–507; 2. Kaczmarek et al. *Genome Med* 2017;9:60; 3. Springer et al. *Nucleic Acid Ther* 2018;28:109–18; 4. Friedrich & Aigner. *BioDrugs* 2022;36:549–71.

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GalNAc–siRNA Conjugates Enable Targeted Delivery to the Liver[1–3]

1. The trivalent GalNAc ligand has a high affinity for the ASGPR, expressed on the surface of hepatocytes[1,2]
2. Upon binding, GalNAc–siRNA conjugates are engulfed into hepatocytes by receptor-mediated endocytosis[1,2]
3. GalNAc and the linker are degraded off the siRNA conjugate and free siRNA passes into the hepatocyte cytoplasm[1,2]
4. Once in the cytoplasm, siRNAs are loaded onto RISC, targeting and degrading the corresponding mRNA, and decreasing production of the target protein[1,2]

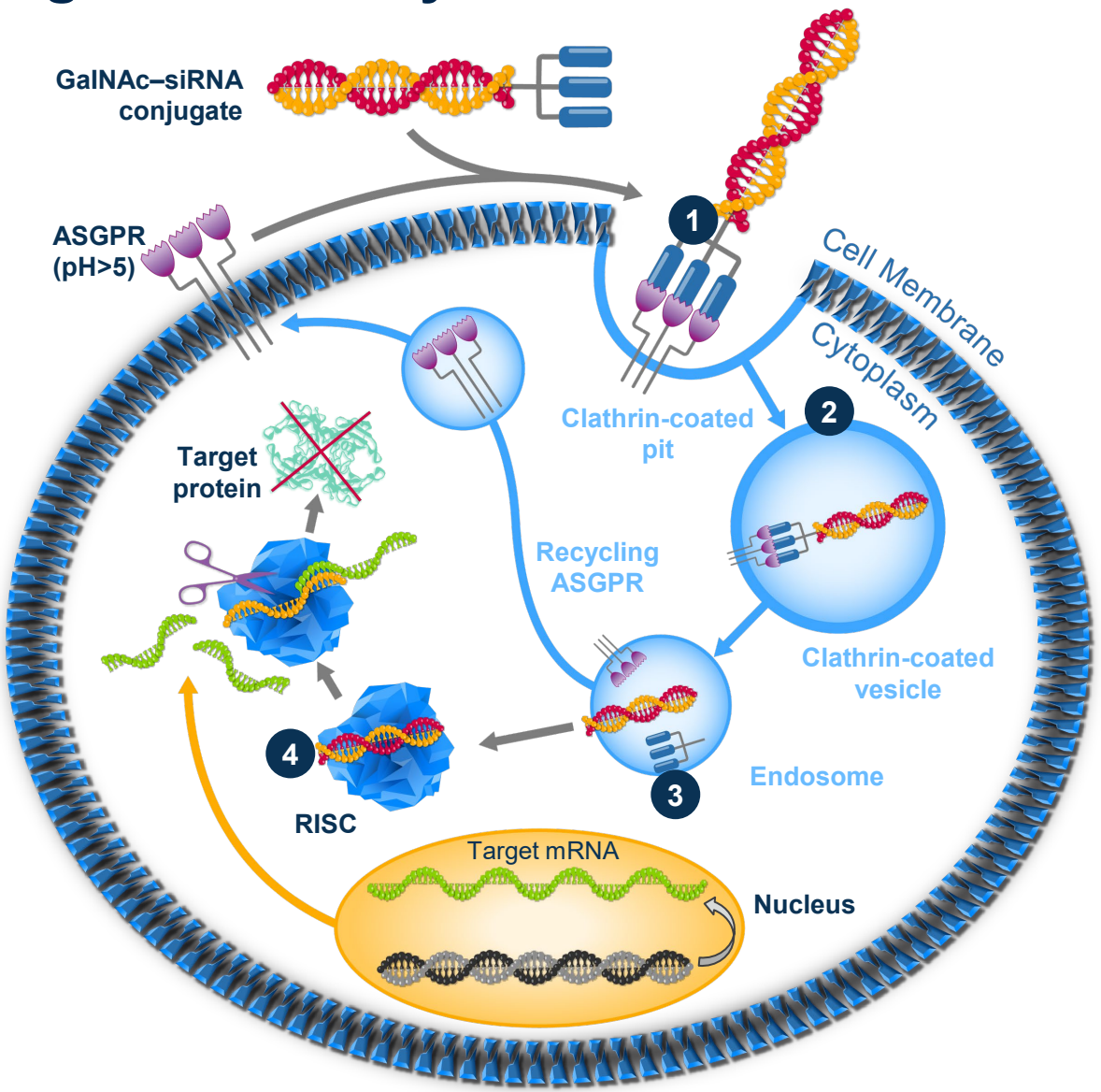

Figure adapted with permission from Benizri et al. *Bioconjug Chem* 2019;30:366–83. Copyright (2024) American Chemical Society[3]

ASGPR, asialoglycoprotein receptor; GalNAc, N-acetylgalactosamine; mRNA, messenger RNA; RISC, RNA-induced silencing complex; RNA, ribonucleic acid; siRNA, small interfering RNA.

1. Springer et al. *Nucleic Acid Ther* 2018;28:109–18; 2. Huang. *Mol Ther Nucleic Acids* 2017;6:116–32; 3. Benizri et al. *Bioconjug Chem* 2019;30:366–83.

Summary

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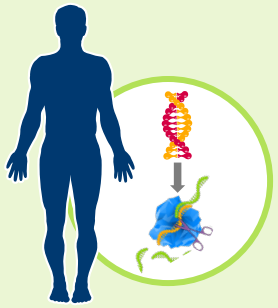
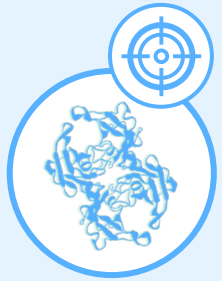
Summary

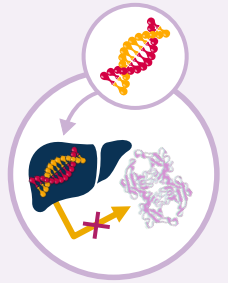
RNAi is a naturally occurring mechanism which cells use to silence gene expression[1–3]



RNAi therapeutics utilize this endogenous mechanism and can be synthesized to silence a specific disease-causing gene[1–5]



RNAi therapeutics can decrease production of the target disease-causing protein[1,4,5]



Delivery of RNAi therapeutics can be targeted to the organ where the protein is being produced, for example the liver[1,4,6]

RNAi, ribonucleic acid interference.

1. Niemietz et al. *Molecules* 2015;20:17944–75; 2. Hu et al. *Signal Transduct Target Ther* 2020;5:101; 3. Chery. *Postdoc J* 2016;4:35–50; 4. Friedrich & Aigner. *BioDrugs* 2022;36:549–71; 5. Jay et al. *Int J Cardiovasc Sci* 2021;35:665–7; 6. Kaczmarek et al. *Genome Med* 2017;9:60.

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RNAi Science

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