RIBONUCLEIC ACID INTERFERENCE: FROM LAB TO BEDSIDE

ST-6

Hello!

I am Tapani Ronni

I am here because I love to give scientific presentations.

You can find me at:
www.polarbearcommunications.com

About the speaker

- PhD in Genetics, University of Helsinki, Finland
- Postdoctoral fellow, University of California, Los Angeles
- Scientific interests: gene therapy, microbiology, immunology
- A full time medical translator since 2007 (English-Finnish)

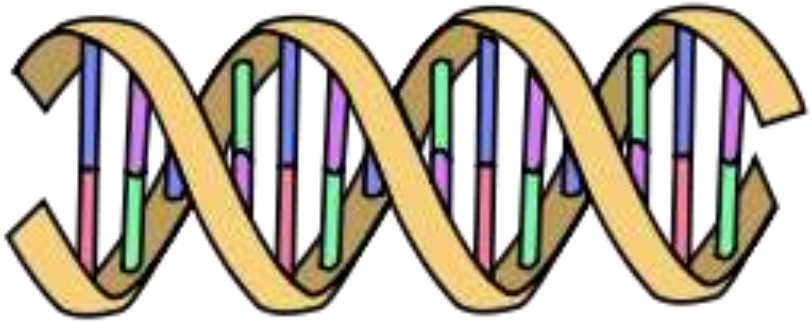
Contents of this talk

- From gene to RNA to protein
- Anti-sense tools for gene regulation
- Introduction to RNA interference (RNAi)
- Gene regulation by RNAi
- RNAi compared to CRISPR
- RNAi in cancer therapy
- RNAi status in clinical trials
- The future of RNA interference

From gene to RNA to protein

A schematic view of DNA structure.

Each of the four bases is shown with different color.



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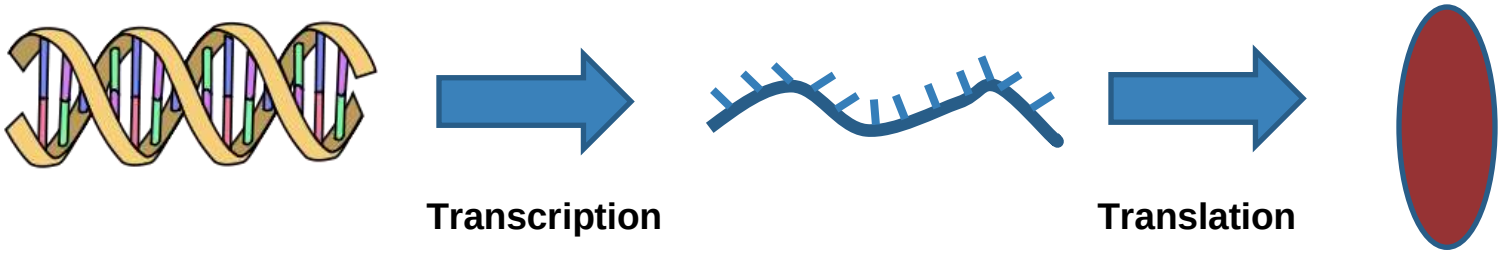
From gene to RNA to protein (continued)



Genomic, double stranded DNA

Single-stranded RNA (*messenger RNA*)

Protein

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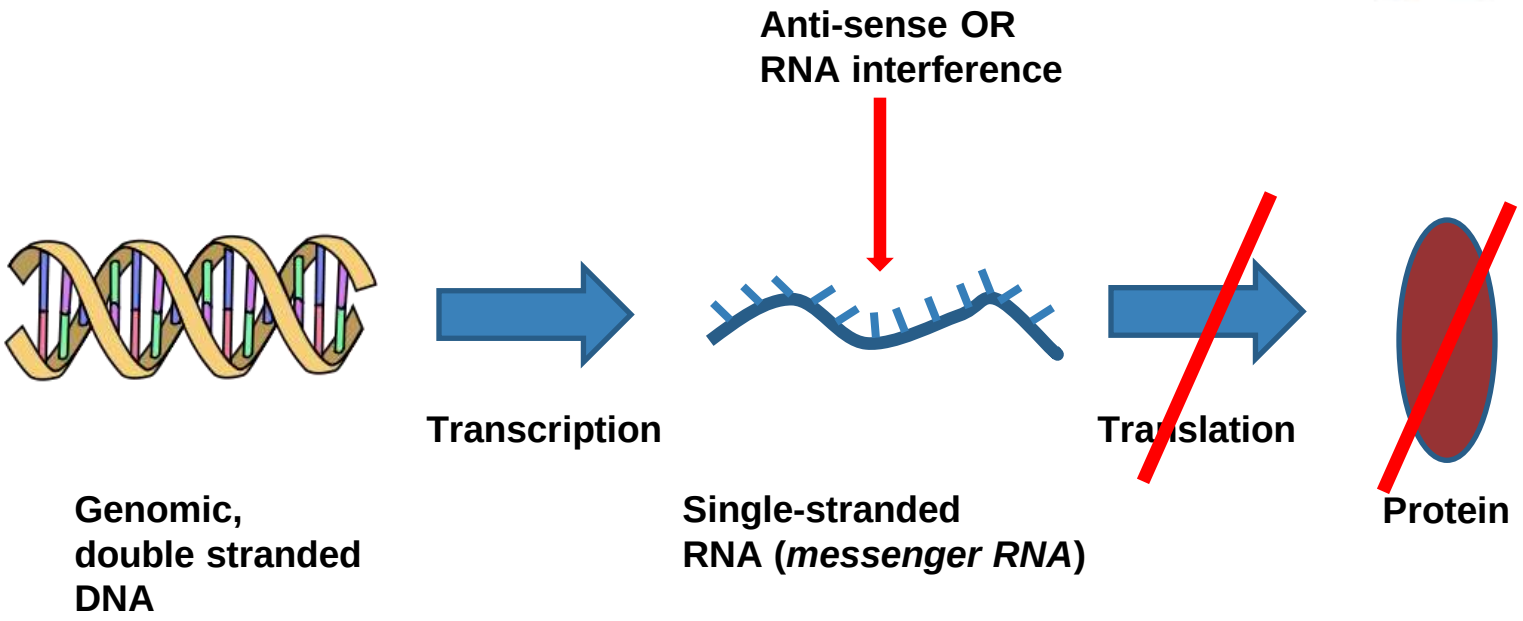
Messenger RNA

- Messenger RNA (mRNA) is produced in the cell nucleus and exported to the cytoplasm
- mRNA is read in *ribosome*
- Ribosome translates the information into a polypeptide sequence
- The new polypeptide is then folded into a functional protein, such as an enzyme
- Proteins may be exported or stay in the cell

Targeting RNA

- Many diseases are caused by faulty proteins
- For example, cancer, inherited metabolic disorders
- Small-molecule drugs have their limitations
- What if you could fix the problem at the source?

Targeting RNA (continued)

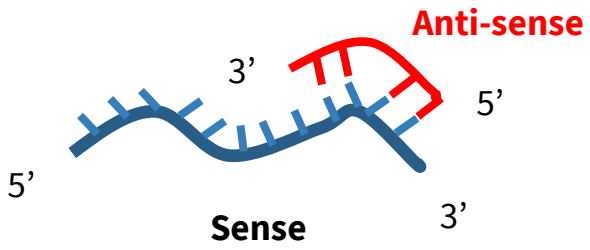


Targeting RNA with anti-sense oligonucleotides

- It is easy to make short DNA strands that recognize the mRNA sequence of interest
- Then the corresponding protein level goes down as translation is blocked
- Anti-sense DNA can also cause the mRNA to be degraded in the cell
- Also, specific protein length can be altered
- Five anti-sense DNA drugs have been approved by FDA to treat various diseases where normal drugs don't work

Targeting RNA with anti-sense oligonucleotides

- Anti-sense oligonucleotide is a short nucleic acid molecule with an opposite polarity than the target nucleic acid
- Therefore, it forms a double stranded form with the target (RNA)
- RNA cannot function and is degraded



Discovery of RNA interference

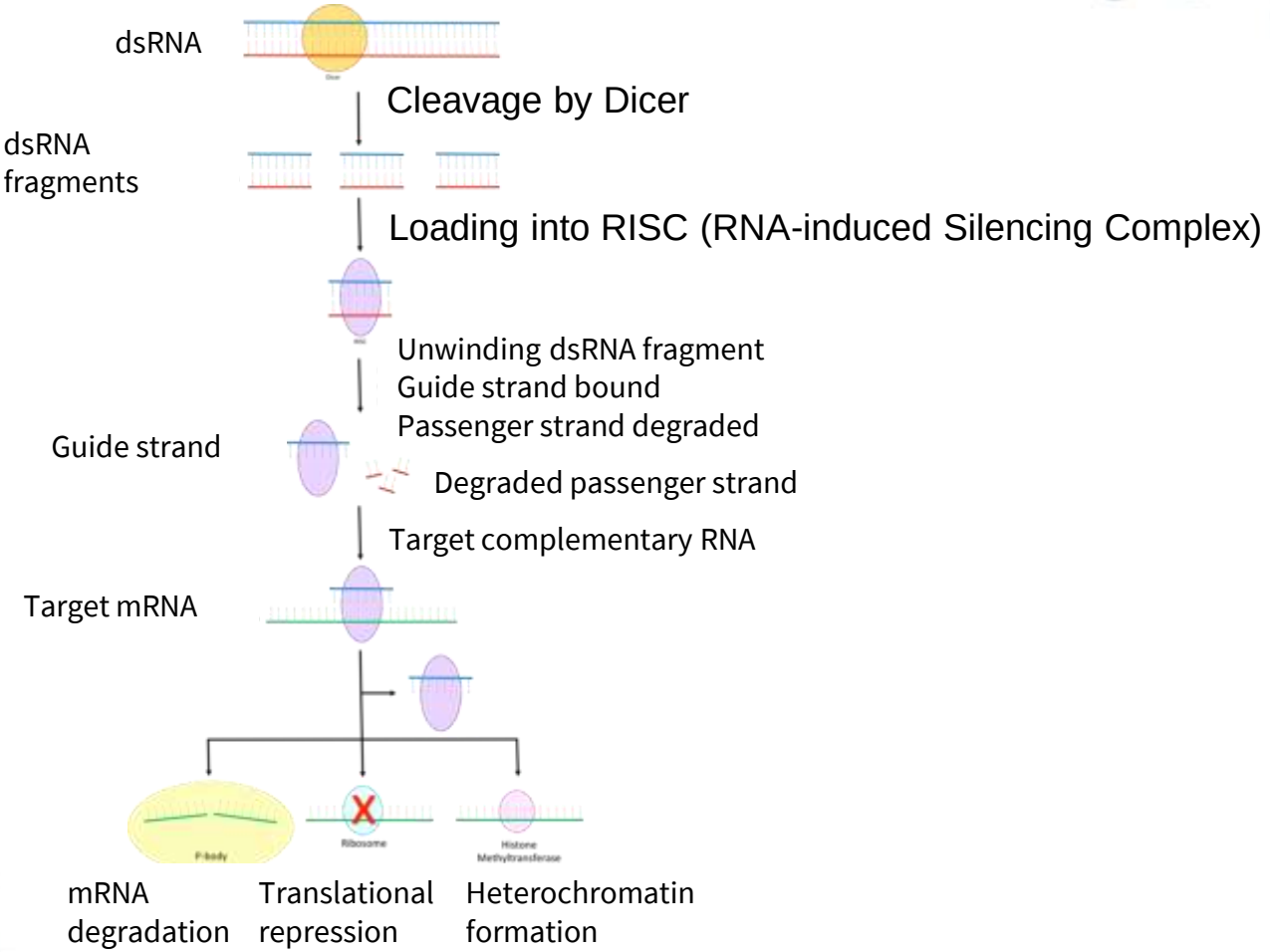
- A landmark *Nature* paper in 1998
- Researchers wanted to interfere with gene expression in *Caenorhabditis elegans* worm using anti-sense molecules
- Good controls led to a serendipitous discovery:
- A short, *single stranded* anti-sense RNA molecule was *less* effective than a *double-stranded* version of the same
- *A few molecules* per worm were enough to suppress target RNA expression
- Nobel price in 2006 to Andrew Fire and Craig Mello

Fire, A et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998 Feb 19;391(6669):806-11

Gene regulation by small RNAs

- RNA interference is a natural phenomenon with its own purpose
- Our genome codes for over 1,000 small RNAs with important regulatory activities
- They are involved in *post-transcriptional regulation* of the genes – they regulate the amount and longevity of mRNAs
- The biochemical mechanisms of micro-RNA (mi-RNA) and small interfering RNA (si-RNA) production are too complex to discuss in this talk

Gene regulation by small RNAs (continued)



RNAi as a research tool

- The original RNAi paper led to furious activity
- Three years later RNAi was shown to work in mammalian cells[1]
- One application: “knock-down” of a gene of interest
  - compare to “knock-out” where the whole gene is deleted
- Today one can buy custom siRNA molecules from vendors
- Knock-down effect <100% but this can be beneficial

1. Elbashir SM1, et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

RNAi as a research tool (cont'd)

Advantages

Speed and efficiency
Cell lines in days
Cheaper than a knock-out
Better mimicry with the effect of real drugs

Disadvantages

Need high efficiency of delivery to target cell
Unspecific side effects caused by siRNA
siRNA can be degraded in the cell
RISC complex can be flooded by excess artificial siRNA

RNAi as a research tool (cont'd)

- As always, careful controls are necessary for good science!

- Repeated experiments with different controls are necessary before making any conclusions

RNAi as a research tool (cont'd)

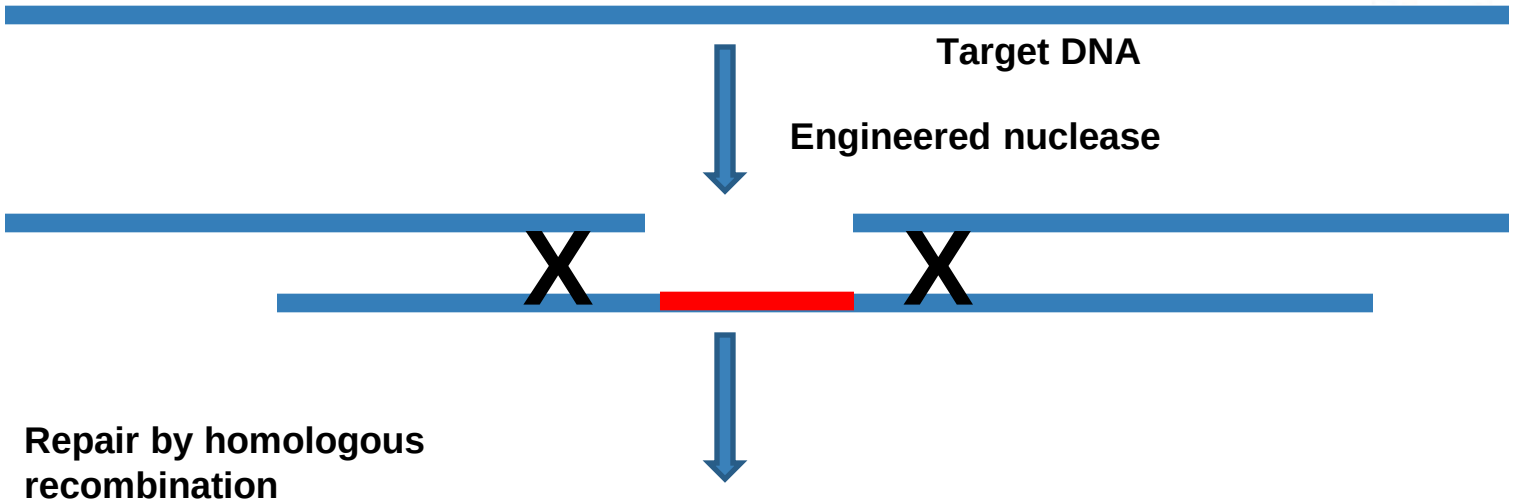
- **BIG DATA biology**

- Genome-wide screening: thousands of siRNAs made to knock down all or most of known genes e.g. in human or mouse cells, or in fruit fly or worm
- Massive amounts of data -> computational approaches and bioinformatics tools needed
- Careful analysis is necessary
- Watch out for false signals, off-target effects, interferon response

CRISPR: an alternative to RNAi

- Microbial defense mechanism against viruses and foreign DNA
- Microbes have to differentiate between ”foreign” and ”self” DNA
- CRISPR system cleaves foreign DNA
  - [CRISPR = Clustered regularly interspaced short palindromic repeats]

Genome Editing using CRISPR



Corrected point mutation (e.g. with Sickle Cell Anemia)

Functional gene correction (e.g. with SCID-X1)

Safe harbor gene addition (e.g. with SCID-X1)

RNAi vs. CRISPR

RNAi

Target is mRNA

“Knock-down”

Effects not always clear-cut

Effects often reversible

Simpler technology

Cheap and fast

CRISPR

Target is DNA

“Knock-out”

Results often clear-cut

Effects not easily reversible

More complicated

Ability to generate permanent cell lines and knock-out / transgenic animals

RNAi vs. CRISPR (cont'd)

- In clinical use, the reversible nature of RNAi is beneficial
- Genome is not edited so if an unexpected adverse event occur, the therapy can be discontinued without permanent harm
- Off-target effects (unintentional editing) of the CRISPR system are still a concern

RNAi in clinical development

- Many “undruggable” targets in biomedicine
- What does this mean?
  - It has not been possible to design a small molecule / biological drug that would potently inhibit or activate a given target protein
  - Target structure may be unknown
  - Even with 3D information known, target surface may be too flat and unspecific

RNAi in clinical development (cont'd)

- One of the leaders in the RNAi field, Alnylam Pharmaceuticals, has compared small molecule drugs to "mopping the floor" while RNAi is like "turning off the spigot" that leaks
- What do they mean?

RNAi in clinical development (cont'd)

- With RNAi one could prevent a dysfunctional protein from forming in the first place
- Some tantalizing options:
- Oncogenes in cancer are often "undruggable" with chemical methods
- With RNAi, these kinds of targets can be "knocked down" in lab and the effect studied *in vitro*
- The most promising siRNA molecules can then be advanced to *in vivo* and clinical studies

RNAi in clinical development (cont'd)

- Very large investments in RNAi discovery work by big pharma during 2000-2010
- Due to lack of success big pharma mostly left the field
- Current leaders in the field are academics and Alnylam Pharmaceuticals (Cambridge, MA)
- Alnylam has been focusing on RNAi for almost 20 years

RNAi in clinical development (cont'd)

- The first RNAi drug candidate nearing FDA approval is **patisiran** by Alnylam
- Patisiran is indicated for a rare disease (50,000 patients world wide) called hereditary transthyretin (TTR) mediated amyloidosis
- Caused by mutations in TTR, causes debilitating symptoms and is often fatal due to heart failure
- Positive phase III results regarding pain and quality of life
- This is not a cure!

RNAi in cancer

- One of the most challenging therapeutic targets
- Many undruggable target proteins
- Solid tumors harder to treat than blood cancers
- Instead of siRNA molecules, better to deliver expression constructs (plasmids, virus vectors) that make siRNA inside the target cell
- Local delivery preferred over systemic delivery
- Better efficacy and safety profile

RNAi in cancer

- Ideal targets would be tumor-specific genes essential for a) the cancer cell survival or b) drug resistance
- a) The proliferation of cancer cells could be stopped or significantly slowed down
- b) Cancer could be more vulnerable to chemotherapy if chemotherapy resistance could be prevented

- Toxicity can be avoided with chemical modifications
- Improved sequence selection -> fewer off-target silencing events

Future of RNAi

- First drug, patisiran, expected to hit the market in 2018
- Many others in clinical development, mostly for rare diseases
- With technological advances RNAi may prove to be a good tool against cancer
- It took 20 years for monoclonal antibodies to move from lab to wide clinical use
- As siRNAs can be developed based on genomic/mRNA sequence alone they can be powerful tools in drug development

Further reading

◎1. Fire, A et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998 Feb 19;391(6669):806-11.
◎2. Ipsaro, JJ, Joshua-Tor, L. From guide to target: molecular insights into eukaryotic RNAi machinery. Nat Struct Mol Biol. 2015 Jan;22(1):20-8. doi: 10.1038/nsmb.2931. Review.
◎3. Elbashir SM1, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.
◎4. Boettcher M, McManus MT. Choosing the Right Tool for the Job: RNAi, TALEN, or CRISPR. Mol Cell. 2015 May 21;58(4):575-85. doi: 10.1016/j.molcel.2015.04.028. Review.
◎5. Mohr, SE, Perrimon, N. RNAi screening: new approaches, understandings, and organisms. Wiley Interdiscip Rev RNA. 2012 Mar-Apr;3(2):145-58. doi: 10.1002/wrna.110. Epub 2011 Sep 22. Review.
◎6. Unniyampurath U, Pilankatta R, Krishnan MN. RNA Interference in the Age of CRISPR: Will CRISPR Interfere with RNAi? Int J Mol Sci. 2016 Feb 26;17(3):291. doi: 10.3390/ijms17030291. Review.
◎7. Conde J, Artzi N. Are RNAi and miRNA therapeutics truly dead? Trends Biotechnol. 2015 Mar;33(3):141-4. doi: 10.1016/j.tibtech.2014.12.005. Epub 2015 Jan 13.
◎8. Wang T, et al. Challenges and opportunities for siRNA-based cancer treatment. Cancer Lett. 2017 Feb 28;387:77-83. doi: 10.1016/j.canlet.2016.03.045. Epub 2016 Apr 1. Review.

Thanks!

Any questions?

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Credits

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