# Patenting Interfering RNA

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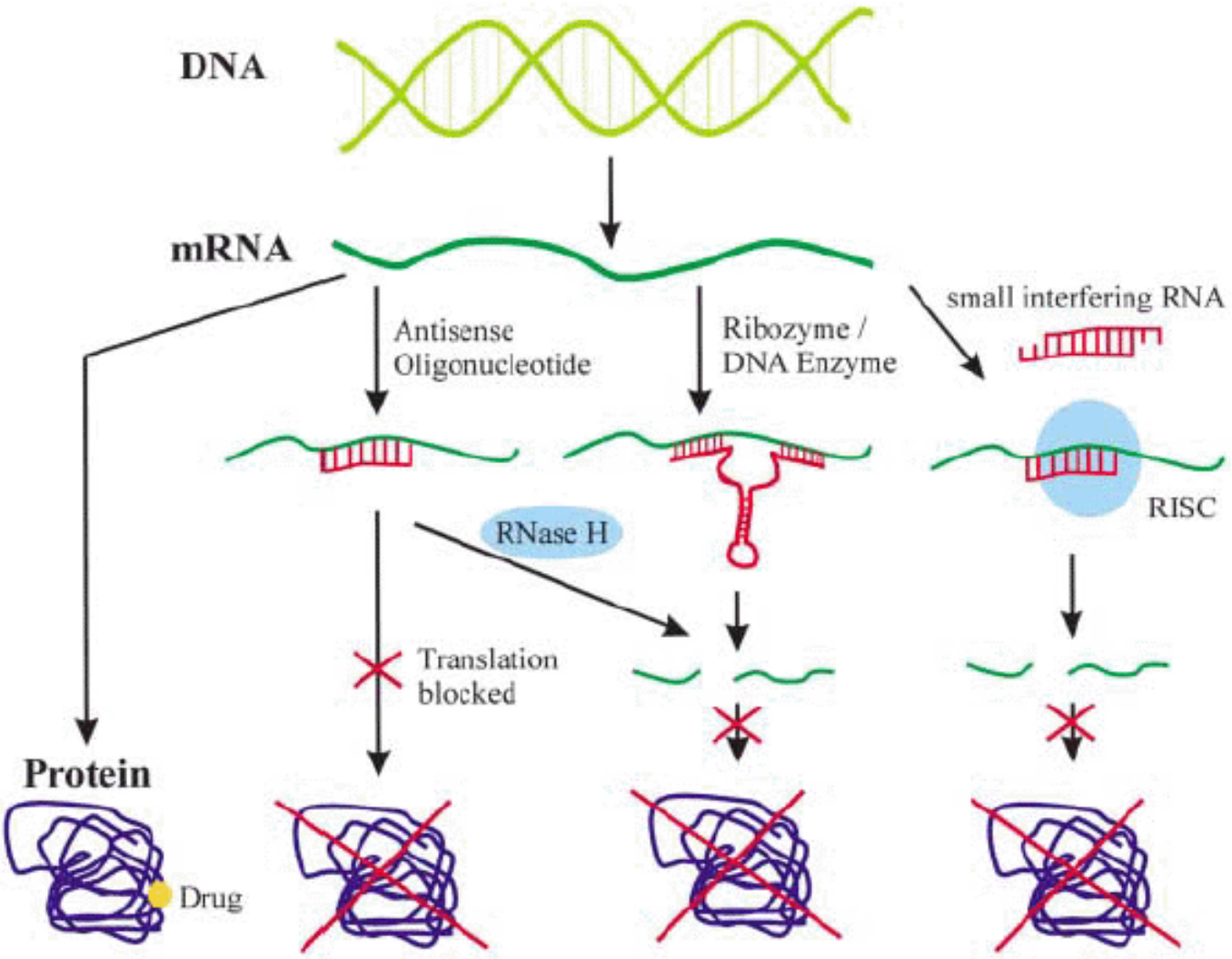
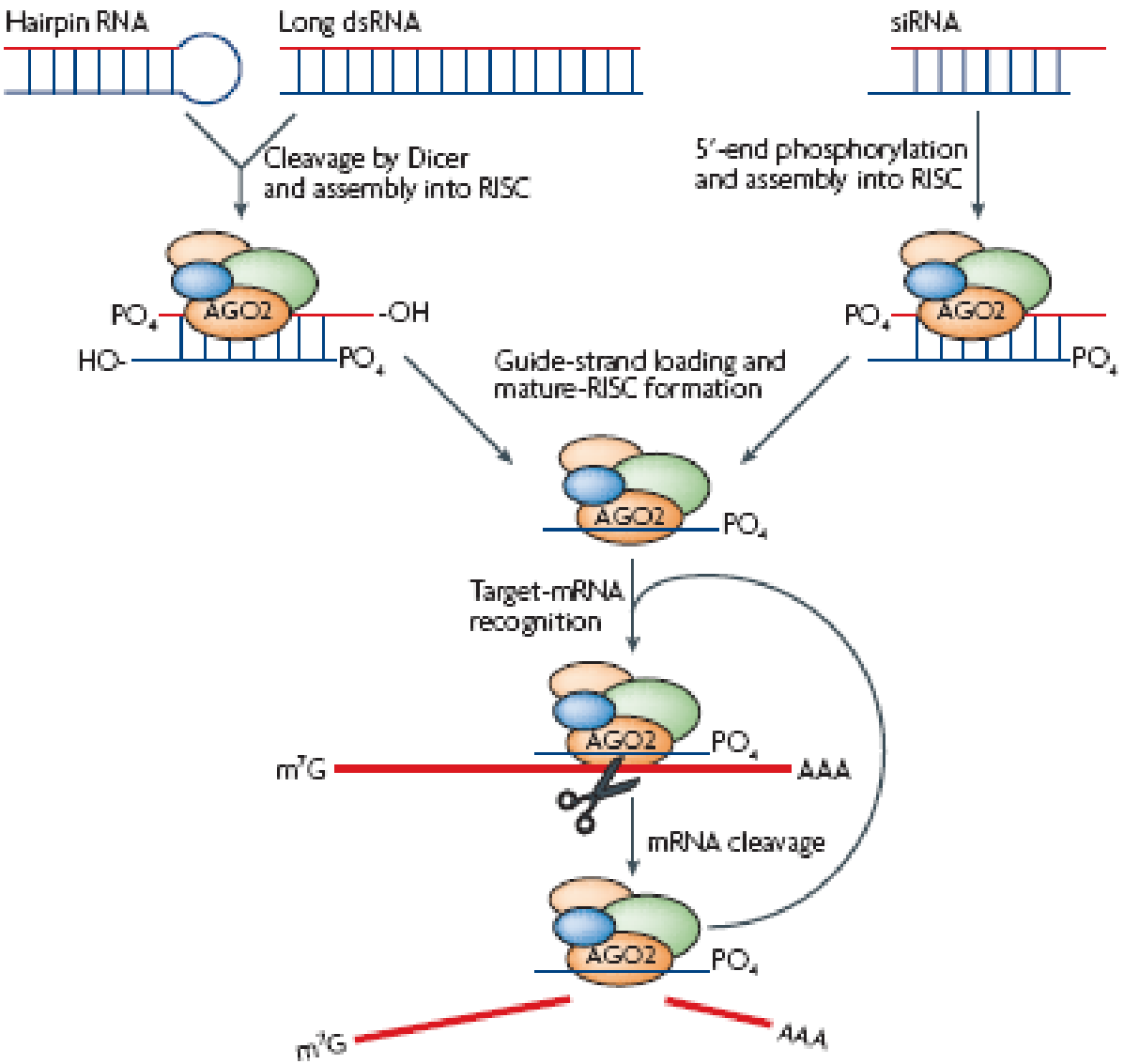
# Oligonucleotide Inhibitors: Mechanisms of Action



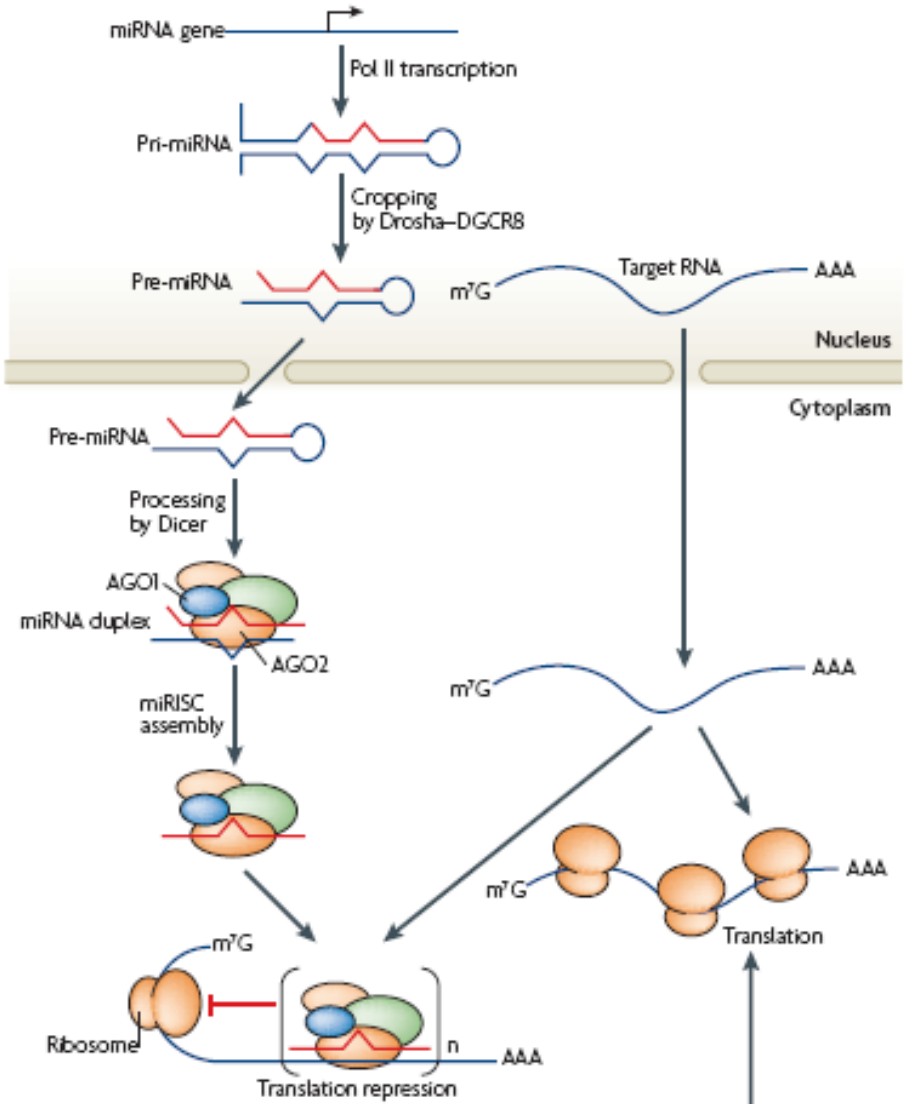
# RNAi - Mechanism of Action

- dsRNA induces sequence-specific degradation of homologous gene transcripts
- RISC metabolizes dsRNA to small 21-23-nucleotide siRNAs
  - RISC contains dsRNase (“Dicer”), ssRNase (Argonaut 2 or Ago2)
- RISC utilizes antisense strand as “guide” to find cleavable target

# siRNA Mechanism of Action



# miRNA Mechanism of Action



# Interfering RNA

## Glossary of Terms

- RNAi – RNA interference
- dsRNA – double stranded RNA
- siRNA – small interfering RNA, double stranded, 21-23 nucleotides
- shRNA – short hairpin RNA (doubled stranded by virtue of a ssRNA folding back on itself)
- miRNA – micro RNA
- RISC – RNA-induced silencing complex
  - Dicer – RNase endonuclease

## siRNA

- Exogenously delivered
- 21-23mer dsRNA
- Acts through RISC
- Induces homologous target cleavage
- Perfect sequence match
  - Results in target degradation

## miRNA

- Endogenously produced
- 21-23mer dsRNA
- Acts through RISC
- Induces homologous target cleavage
- Imperfect sequence match
  - Results in translation arrest

# RNAi Patentability issues

Sample Claims:

- *A siRNA that inhibits expression of a nucleic acid encoding protein X.*

OR

- *A siRNA comprising a 2'-modification, wherein said modification comprises 2'-fluoro, 2'-O-methyl, or 2'-deoxy. (Note: no target recited)*

OR

- *A method of reducing tumor cell growth comprising administering siRNA targeting protein X.*

# RNAi Patentability Issues

## 35 U.S.C. 101 – Utility

- Credible/Specific/Substantial/Well Established.
- Used to attempt modulation of gene expression in human diseases
- Routinely investigate gene function in a high throughput fashion or to (see Rana RT, Nat. Rev. Mol. Cell Biol. 2007, Vol. 8:23-36).
- Knowledge of gene function sufficient to warrant target inhibition is required to have Utility.

# RNAi Patentability Issues

## 35 U.S.C. 101 – Utility

- If no function for target nucleic acid (protein or regulatory) is in evidence:
  - siRNA/miRNA processes would likely lack utility
    - siRNA used to probe function of gene with unknown function is not sufficient to provide utility for siRNA/miRNA
  - May raise enablement (how to use) and/or written description issues

## 35 U.S.C. 112, first paragraph, Enablement

# RNAi Predictability

- Bioinformatic screening effective to narrow candidate siRNA's
  - Can greatly reduce number of screens to find active siRNA's
  - Takes into account a number of "targeting rules" identified by researchers
- Long dsRNAs cause severe sequence-non-specific effects
  - induces apoptosis from shut down of translation
  - Small size of ~ 21nts required to avoid most effects

## 35 U.S.C. 112, first paragraph, Enablement

# RNAi Predictability

- High *in vivo* unpredictability due to general lack of knowledge regarding efficacy and *in vivo* target site determination, and delivery issues, methods particularly.
- Delivery, Delivery, Delivery
- To date only one human antisense with FDA approval.
  - no FDA approval for any siRNA, miRNA, ribozyme, etc.

# RNAi Patentability Issues

## 35 U.S.C. 112, first paragraph, Written Description

- Possession of genus depends upon description of a representative number of species.
  - In the case of a small genus covering a limited defined target or siRNA/miRNA, one species may be representative.
    - identify all relevant distinguishing characteristics relating to the scope of the claims.
    - identify all elements claimed and their support in the description
- Art-recognized structure/function relationship.
  - identify species explicitly or implicitly disclose
- Reconcile with the level of skill in the art.

# RNAi Patentability Issues

## 35 U.S.C. 112, first paragraph, Written Description

- siRNA/miRNA described only by function may lack written description.
- Claim 1. *A siRNA that inhibits expression of a nucleic acid encoding c-raf.*
  - What is the size of genus embraced by the named gene?
  - Does it include functional fragments, homologues, alleles, etc.?
  - What species are described in spec/prior art?
  - Description may be considered complete if target ID'd by SEQ ID NO:.

# State of the Art

- Today, probability of finding a single, individual functional siRNA/miRNA out of a genus is high.
  - A broad claim to ***"An isolated siRNA that inhibits the expression of human gene X."*** may be enabled/described by providing the sequence for gene X.

- Today, predictability of any single siRNA being effective varies greatly depending upon target, but overall is thought to be about ~50%.
  - Requires modern bioinformatic screening first

- Going back in time, Enablement and Description issues generally increase, since they are analyzed for the state of the art at the time of filing, and since this art is very new.

# RNAi Patentability Issues

## 35 U.S.C. 112, first paragraph, Written Description

- Written Description Conclusions:
  - Broad claims to siRNAs inhibiting expression of a nucleic acid encoding a protein may lack an adequate written description.
    - Provide evidence that target one sequence correlates with targeting other versions of the gene.
  - As a rapidly evolving field, Enablement and Written Description issues become complex since they are analyzed for the state of the art at the time of filing.
    - The more you show and/or is known, the more you can possibly claim.

# RNAi Patentability Issues

## 35 U.S.C. 102 – Novelty/Anticipation

- Anticipation of specific siRNA/miRNA
  - must be explicitly taught in the prior art for anticipation to be applicable.

# RNAi Patentability Issues

## 35 U.S.C. 103 - Obviousness

- **Why RNAi may be obvious**
  - Used to routinely to attempt modulate gene expression in human diseases or in cells.
  - Used to investigate gene function.
    - Provided the target is identified in the prior art as desirable for silencing (disease gene, virus).
  - Neither necessarily identifies any specific siRNA sequence.

# RNAi Patentability Issues

## 35 U.S.C. 103 - Obviousness

- **Expectation of Success**
  - expectation of RNAi gene silencing highly likely for target sites identified as accessible to antisense inhibition (see Vickers et al. (J. Biol. Chem.) 278: 7108-7118, 2003).
    - in vitro
  - low expectation of success for *in vivo* applications.
  - High expectation of success in identifying specific modifications that are tolerated
    - Use of high-throughput assays are routine, and modification chemistry known.

# RNAi Patentability Issues

## 35 U.S.C. 103 - Obviousness

- Obviousness rejections may be proper against genus siRNA/miRNA claims to a known gene sequence if the prior art suggested its inhibition by nucleic acid-based or other methods.

*Claim: A siRNA that inhibits expression of a nucleic acid encoding protein X.*

  - Antisense and ribozyme art may apply against this claim, given their art-recognized relationship.
  - Narrow claims to specific RNAi sequences may be free of the art.

# RNAi Patentability Issues

## 35 U.S.C. 103 - Obviousness

- Obviousness rejections may be proper against broad RNAi claims reciting no target and limited only to a specific, known chemical modification.

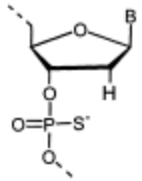
*Claim: A siRNA comprising a 2'-modification, wherein said modification comprises 2'-fluoro, 2'-O-methyl, or 2'-deoxy.*

  - Analysis: siRNA compounds are known generally, the modification is known to confer benefits, and high throughput assays to test efficacy are well known in the art.

# Common Nucleotide Modifications:

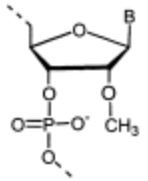
Confer nuclease resistance, enhance binding

**First generation**

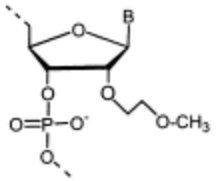


Phosphorothioate DNA
(PS)

**Second generation**

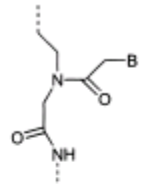


2'-O-methyl RNA
(OMe)

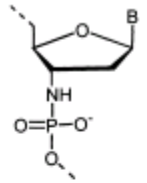


2'-O-methoxy-ethyl RNA
(MOE)

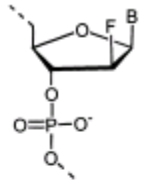
**Third generation**



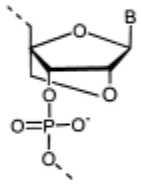
Peptide nucleic acid
(PNA)



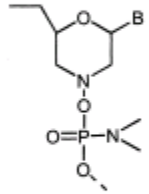
N3'-P5' Phosphoroamidate
(NP)



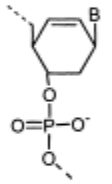
2'-fluoro-arabino nucleic acid
(FANA)



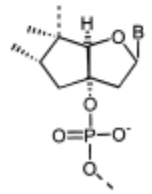
Locked nucleic acid
(LNA)



Morpholino phosphoroamidate
(MF)



Cyclohexene nucleic acid
(CeNA)



Tricyclo-DNA
(tcDNA)

# Recommendations

- Claim functional siRNA by specific sequence.
- List results of any siRNA/miRNA compound tested
  - Such “gene walk” data may provide representative number of species for broad scope of a generic claim.

# Recommendations

- Provide *objective evidence* that *in vitro* results are ***representative*** of *in vivo* applicability.
- Respond to examiner-cited unpredictable factors with *objective evidence* to the contrary.
- Expert opinions are more favorably viewed when supported using *objective evidence*.
- Provide *objective evidence* that a particular animal model is generally accepted as ***representative*** of disease or methods of treating, particularly for humans.
- ***Objective evidence*** includes arguments, case law, journal articles, and experimental data and comparisons commensurate with the disclosure as filed.

# RNAi

Questions?