Main Ideas

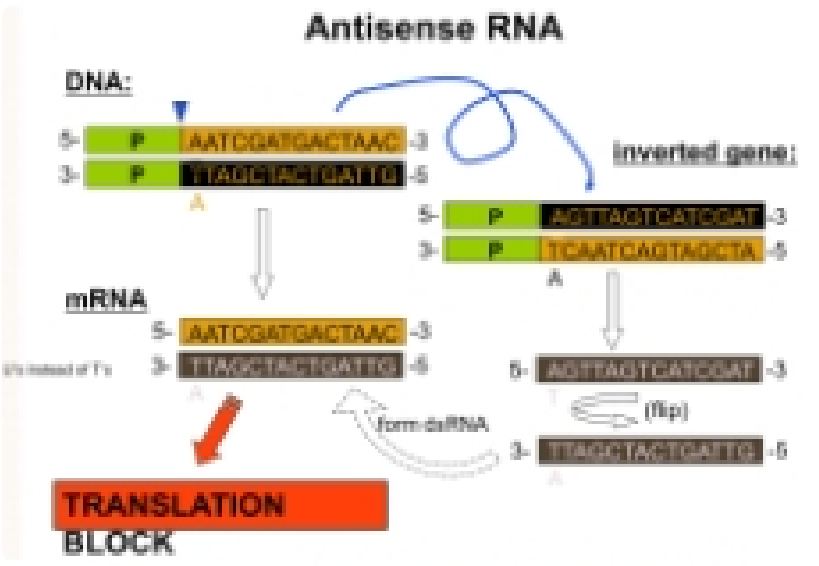
- Principle of RNA interference: use dsRNA to generate small ssRNA molecules that interfere with gene expression
- The two most well known RNAi mechanisms involve:
  - siRNA: generated from exogenous dsRNA sources, causes destruction of the target mRNA molecules
  - miRNA: generated from endogenous miRNA genes; it can cause mRNA destruction or translation inhibition
- siRNA and miRNA differ mostly in how they were first made (biogenesis); the final steps are the same: processing by dicer, assembly into RISC, etc.
- Additional interfering RNA types are still being found
- MicroRNAs are involved in many vital processes in our body cells
- Misregulation of microRNAs is linked to many human diseases, incl. cancer, neuropsychiatric disorders, susceptibility to viral infection
- RNAi is a useful tool in medicine and currently being explored for treatment of various human diseases

Objectives

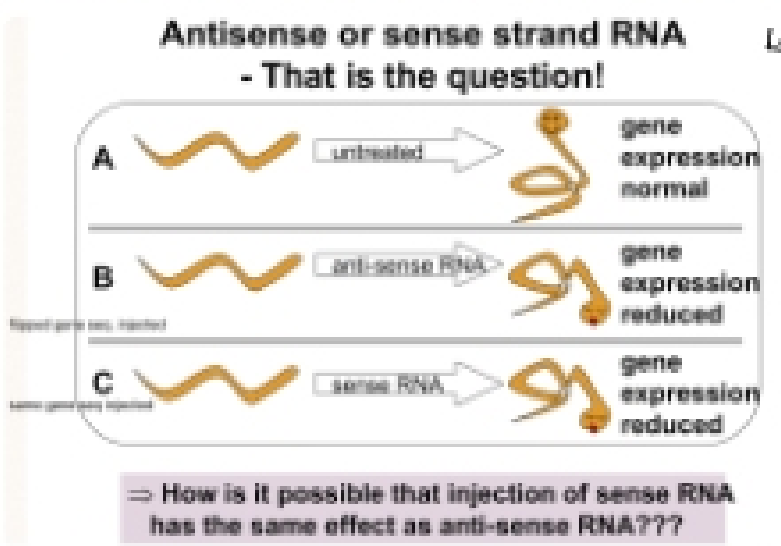
1) Understand the principles of RNA interference
2) Explain the different mechanisms of RNAi-mediated gene silencing
3) Describe the biological roles of RNA interference
4) Explain how microRNA contributes to disease
5) Explain applications of RNAi in treatment of disease

1) Understand the principles of RNA interference

- Interfere with gene expression on the RNA levels (aka stopping that RNA from being translated into a protein) via: antisense RNAs, microRNAs, and small interfering RNAs
- Antisense RNA:
  - artificially "flipped" DNA. It is the mRNA that is the exact complement of normal mRNA-->they bind-->idea is that it interferes with getting the normal mRNA translated into proteins-->this is what happened! Cells with the antisense RNA made a lot less of that specific protein.
- Phenom of cosuppresion: introduce a transgene (adding another gene for purple), you think you'd get a deeper purple, BUT you actually get suppression of gene expression. Sim. results in C. elegans.



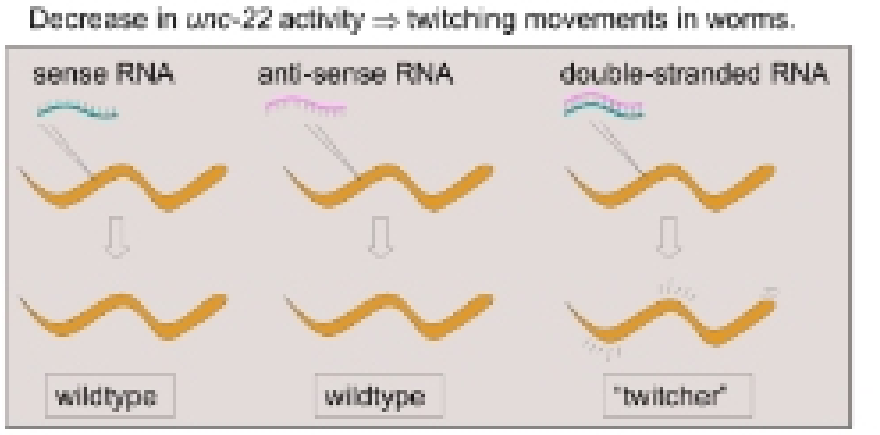
Ignore the beginning of the strand, you actually flip the bottom black part-->make mRNA-->second flip is showing you how it bp with the normal RNA.



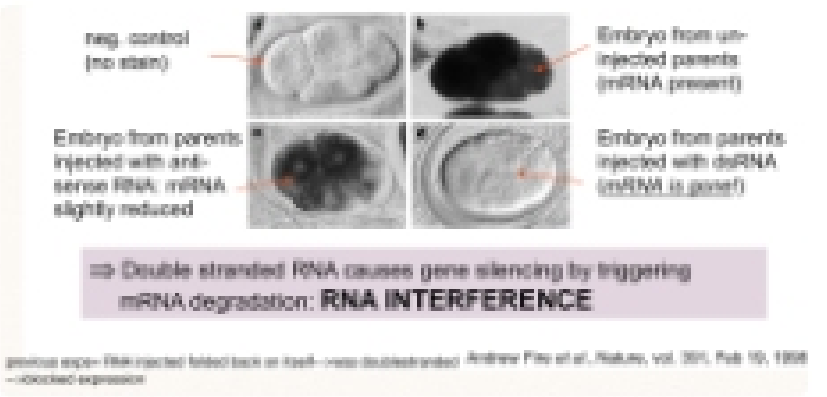
The beautiful "Fire and Mello experiment"

Target: unc-22 gene, encodes for a myofilament protein.

Decrease in unc-22 activity ⇒ twitching movements in worms.



- **Fire and Mello exp. findings**
  - only injection of sense AND anti-sense RNA causes the predicted phenotype. Just injecting sense RNA or anti-sense RNA didn't do it.
  - injection of dsRNA-->triggered degradation -->**loss of the targeted mRNA**

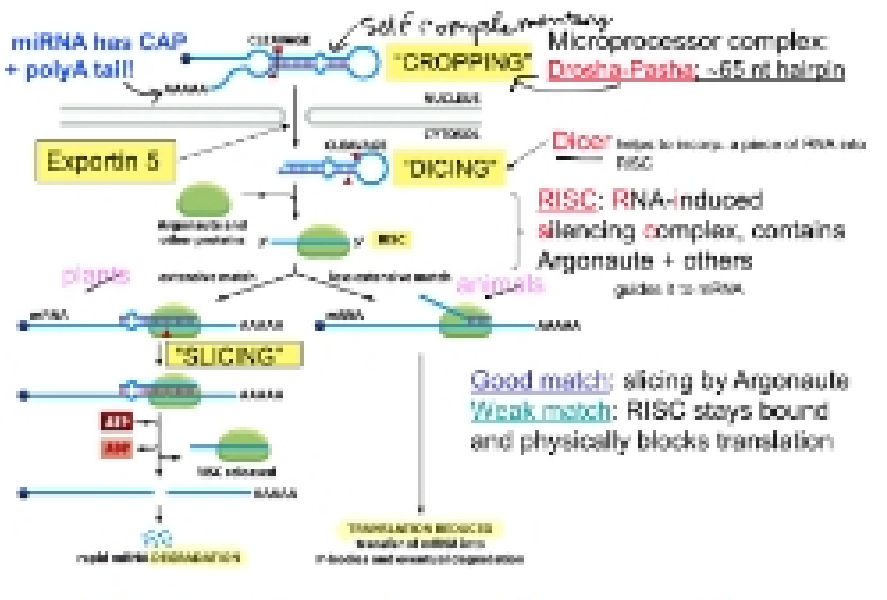


- **Main Idea of RNA interference**
  - cells use RNAi to determine the stability of mRNA or determine accesibility of mRNA for translation machinery
  - dsRNA is chewed up-->small ssRNA pieces-->one strand of dsRNA incorporated into a protein complex-->thru the ssRNA piece, the complex binds to target mRNA (w/complementary seq.)-->targeted mRNA is A. cut by an endonuclease and degraded B. Blocked for translation by bound complex C. Destabilized by the bound complex and degraded.
- **Small interfering RNAs= siRNAs**
  - gen. from exogenous sources (viruses, lab)
  - produced in the cytoplasm by Dicer (RNA pol III)
  - mediate mRNA cleavage
- **microRNAs= miRNAs**
  - encoded in our genome and often transcribed by RNA pol II, you get a ssRNA precursor that forms step loops (= dsRNA sections)
  - Drosha (RNaseIII), then nuclear export and processing by Dicer
  - mediate mostly translational blockage and mRNA destabilization

2) Explain the different mechanisms of RNAi-mediated gene silencing

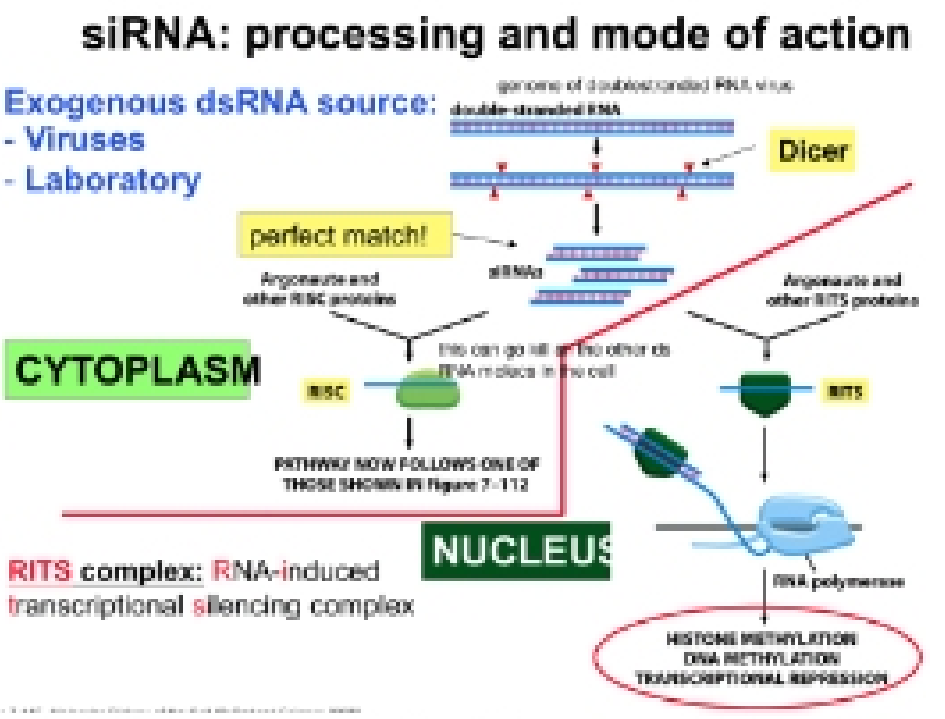
- **miRNA** is cropped by Drosha-Pasha, it is exported out of nucleus, Dicer helps to put a portion of it into RISC, goes good match or weak match route
  - RISC-bound mRNAs are transported to the **P (rocessing)-bodies**-->final destruction of most mRNA
  - each miRNA can repress several diff. mRNAs
  - many mRNAs have many miRNA binding sites
  - humans have 500-1000 miRNA genes, target 40% of our mRNAs
  - miRNAs often target 3'UTRs
    - many roles of miRNAs! embryo dev., anti-viral defense, cell and tissue diff...more diff...more

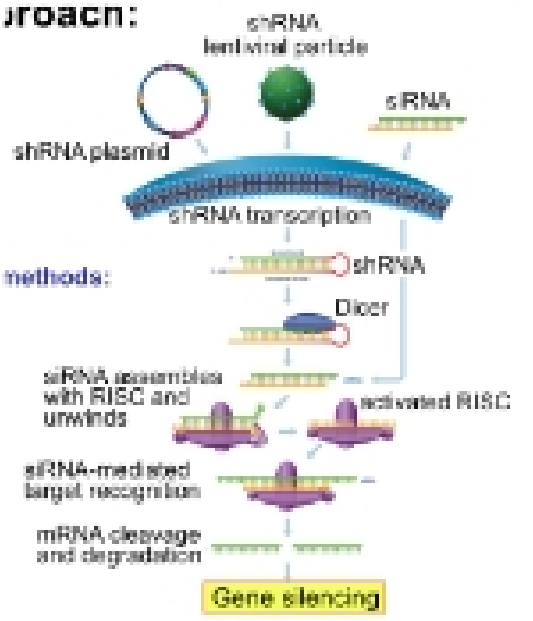
miRNA: processing and mode of action

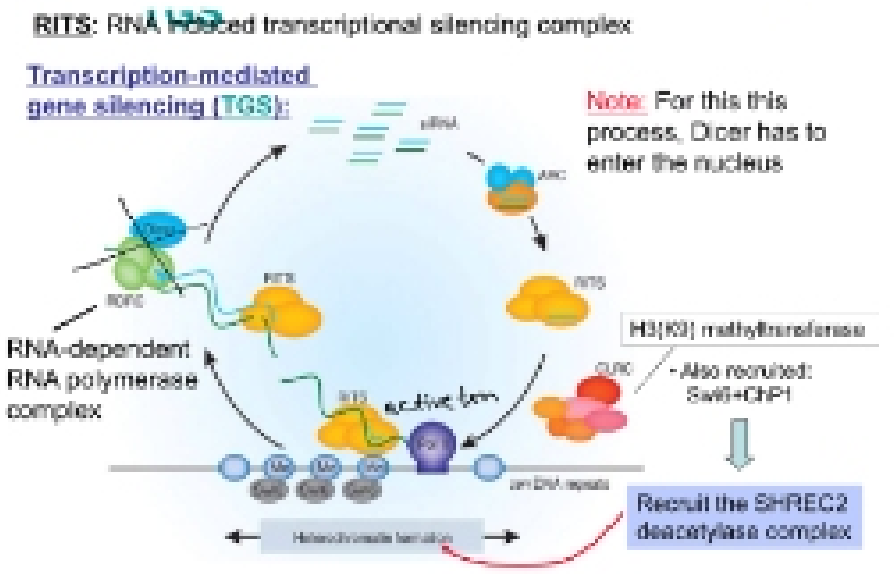


Note: Dicer recognizes ONLY dsRNA

-**siRNA**: get source of dsRNA-->dicer cuts it to make siRNAs-->perfect match-->becomes incorporated into RISC-->follows one of the pathways above
- siRNA: defense against viral infections
    - viral genomes are made of DNA or RNA
    - many RNA viruses produce dsRNAs as intermediates or products of replication
    - many DNA viruses generate dsRNA from convergent transcription of their genome
    - dsRNA is a signature molecular feature of virus infection!!! (in our cyto there isn't dsRNA, we have it in the nucleas with miRNA though)
- **siRNA/shRNA approach**
    - great way in the lab to target and knock down specific genes
    - can use diff. delivery methods:
        - synthetic siRNA
        - shRNA plasmid
        - shRNA lentiviral particle
- **RITS complex**
    - RNA induced transcriptional silencing complex
    - this is in the nucleus, dicer doesn't cut in the nucleus, RITS will recruit modifying enzymes that silence the chromatin behind the gene being transcribed= like a feedback loop!







3) Describe the biological roles of RNA interference
- Viruses, see above

4) Explain how microRNA contributes to disease
- MicroRNAs are involved in a lot of cellular processes
- Function can be affected in a lot of different ways: change in nucleotide sequence by DNA mutation or by RNA editing (ADAR enzyme), defected in miRNA txn, defected in miRNA prcoessing, abnormal targeting
- Abnormal mRNA expression is linked to diseases!
    - Psych and neurodevelopmental disorders
        - ex. increase in DGCR8 expression (gene for Pasha)-->downreg. of synaptic proteins-->lower function of synaptic signaling
    - Viral diseases