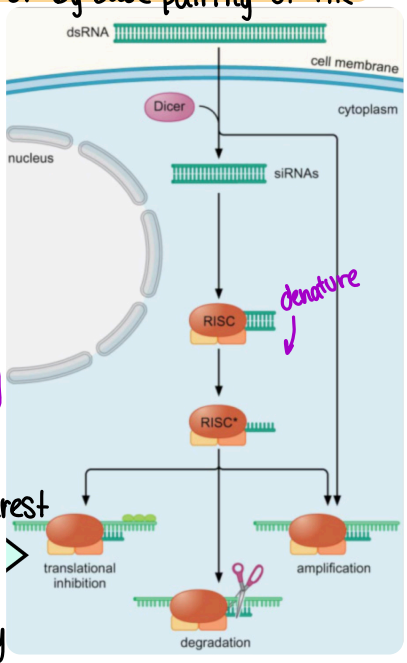
- Andrew Fire and Craig Mello found that they could strongly & reversibly inhibited by introducing a double-Stranded RNA with a base sequence from target gene
  ↳ Sequence is homologous to the genes RNA
- Found embryos injected w/ a single stranded RNA complementary to a particular mRNA had reduced gene expression
  ↳ If embryo injected w/ double stranded RNA expression was effictevely eliminated

* This effect has been reproduced in eukaryotes (not prokaryotes) are called RNA interference (RNAi)
  ↳ RNAi is result of transient destruction of the gene's RNA but does not damage the gene itself so RNAi produces Knockdown (not knockout) of gene expression. This allows us to study function of genes which a permanent knockout would be lethal

- RNA interference is activated when double stranded RNAs either enter the cell from outside or by base pairing of the cells own RNA

① First an enzyme called Dicer which cleaves the ds RNA randomly into 22 Bp fragments
  ↳ 22 Bp fragments of dsRNA are called small-interfering RNA's (siRNA's)
  - siRNA also used to refer to manmade dsRNAs of similar size

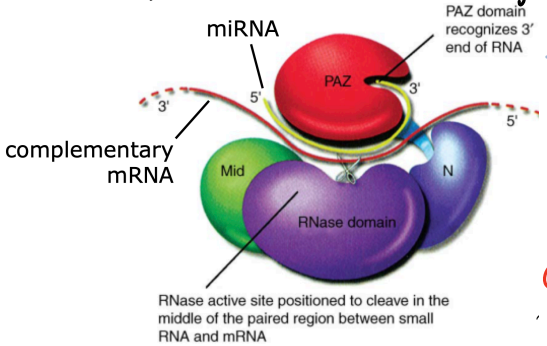
② Actual pathway requires 22 bp siRNA with a protein complex called RISC ↷
  1. First Risc binds mature double stranded SiRNA (RNA Induced Silencing Complex) causing it to denature
    ↳ 'Guide' Strand of siRNA targets RISC to RNAs that contain a Complementary Sequence leading to their inhibiton (the 2nd 'passanger' Strand is degraded)
    - When RNASE & denature sequence combine to target it's degraded or change RNA
  - 2 strands of Si RNA ① Guide b/c has homology to a gene or Sequence of interest
    ② Passanger is degraded at this step



RNA Interference of gene expression can occur at multiple levels

① Translational Inhibition the siRNA : RISC complex can bind to complementary mRNA and prevent translation
  ↳ The complex prevents mRNA strand from associating w/ ribosome so are not translated

② Degredation the siRNA : RISC complex can cleave and degrade the target mRNA
  ↳ The complex will bind to RNA of target gene and bind ss RNA so degraded



RISC is large complex containing proteins one is an RNase called Argonaute
- Argonaute has a Paz Domain that recognizes the 3' end of guide siRNA which allows it to position target mRNA so it can be cleaved by Argonaute's RNase domain
  ↳ The free 5' end binds to target sequence since complementary the Argonaute holds mRNA in place

③ Amplification the siRNA : RISC complex can amplify the target mRNA can be used as template for producing more siRNA molecules
  ↳ complex goes to target mRNA and clips siRNA out of mRNA which can now be used to make a second ds SIRNA which can be used by another complex to target an RNA

Evolutionary History of RNA interference

- SiRNA pathway (Dicer Argonaute) found in most euk & thought to be defense mechanism against RNA virus
  ↳ supported by mutations in genes encoding Dicer or Argonaute not being able to disrupt resistance to viral infection
- Animals & plants synthesize their own double-stranded regulatory RNAs called micro RNAs (miRNAs)
  ↳ Si RNA made expiremental outside of cells & miRNA's are encoded by genome cell itself

MiRNA

- miRNA does not inhibit transcription they effect RNA stability or ability of RNA to be translated
- miRNA is not protein coding only RNA producing
- miRNA are regulated like protein but goal is to modify and regulate expression of other genes making protein by down regulating the expression of other protein coding genes

* Micro RNAs important for humans we encode >5000 miRNA w/ unique sequence, Over 60% protein coding genes regulated by one or more, Mutations can cause diseases (tumor repressant miRNA loss function)

How do cells synthesize microRNAs (miRNA)

① miRNA's are encoded in genome unlike Si RNA they're functional components of organism's genome

② Like messenger RNAs miRNA genes are transcribed by RNA Pol II and the primary transcript and is both capped and polyadenylated

* Hairpin structure forms miRNA

miRNA is capped & polyadenylated

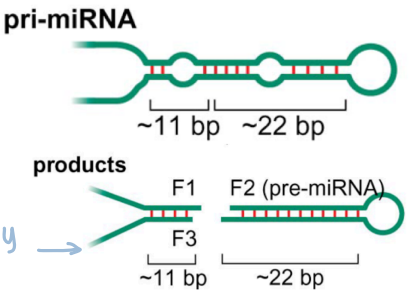
However

③ some miRNAs are transcribed from their own unique genes

④ Other miRNAs are located within the introns of protein coding genes. They're processed after introns are spliced out of pre-mRNA (are transcribed & regulated along protein coding gene till spliced)

- Each functional miRNA arises from a stem loop in secondary structure of the primary RNA transcript
  - ↳ Are cut from primary transcript by subsquental action of 2 ribonucleases RNases that recognize specific structure

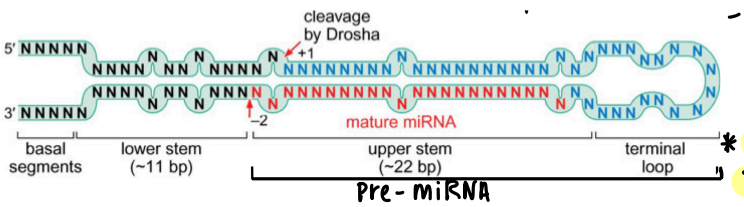
RNases that cut miRNA



used only for recognition

① Drosha cleaves the pri-mRNA within the nucleus (is nuclear enzyme)
- Drosha binds w/ DGCR8 and they hold stem loop in place and cut at base of 11 bp sequence b/c structure recognizes 11 & 22 Bp
- Drosha cleaves a single phosphodiester bond on either side of the stem loop which would create pre-miRNA

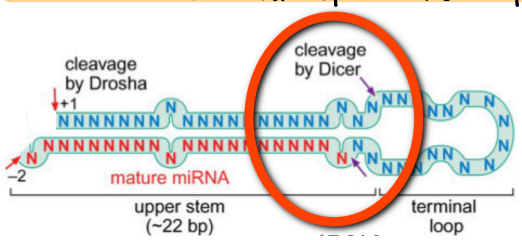
* Cleavage site is determined by shape of stem loop not base sequence
  - ↳ Have improper base pairing which create bulges that are necessary for cutting recognition for miRNA and seperates them into upper and lower stems if nascent miRNA
  - ↳ Cleavege by Dorsha leaves a 3' overhang of 2 nucleotides at base of pre-miRNA shown by arrows



- Pre-miRNA is then transported to the cytoplasm, where its terminal loop is cleaved by second RNase Dicer (same Dicer used in Si RNA but here site not random)

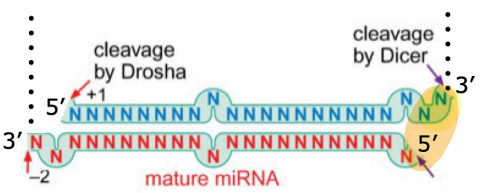
* Big difference between SiRNA and miRNA is miRNA is produced in nucleus and needs to be processed by Dorsha before transported to cytoplasm to be cleaved by dicer

- Dicer removes terminal loop to leave 22 bp sequence



- Dicer has region called Paz Domain that recognizes 3' overhang of pre-miRNA. It will then measure ~22 bp from Dorsha cut site & cleaves phosphodiester bonds at those 2 locations

* Paz domain is structurally homologous to Paz Domain in Argonaute (are related protein but one is nuclear & other cytoplasmic)
  - ↳ Paz Domain in Dicer is cytoplasmic

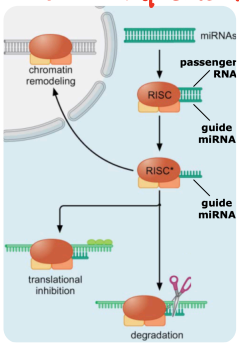


- Dicer leaves 3' overhang that is 2 nucleotides in length

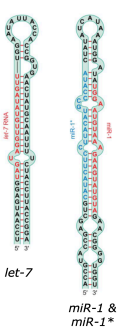
* Both Drosha & Dicer process stem loop on shape so essentially no limit on miRNA base sequence
  - ↳ gives large range of sequences you can target

* like SiRNA mature miRNA can associate w/ RISC & can inhibit translation or cause degradation

Unlike SiRNA, miRNA have sequences that match specific protein coding mRNA's So are more specific to one or few complementary genes



- RNA interference involves miRNA and target sequence base pairing
  - ↳ Not all base pairing is identical in all positions & it can have bubbles form due to nonperfect
- miRNA lined up on 3' UTR of line target gene, multiple miRNA can bind to target and will be attacked & digested
- For many miRNA the guide strand & passanger strand are 2 sides of loops
  - ↳ However for some miRNAs either strand can be used as guide strand. Strands have different base pairs so can inhibit different target mRNAs (due to antisense)



- can have Chromatin Remodeling where miRNA: RISC complex enter nucleus & silence transcription of gene that target mRNA originates
  ↳ Include base pairing between miRNA and the pre-mRNA before transcription is complete

Both have
Paz Domains
RISC
Argonaute
Dicer

miRNA
Drosha → only found in nucleus so only process miRNA and not siRNA

- is cut by Argonaute when miRNA goes to cytoplasm

- Drosha is in nucleus & miRNA is produced in the nucleus not siRNA which is produced by Dicer's cleavege of dsRNA in cytoplasm

Pi RNAs
- animals have piRNAs which are short regulatory RNAs
① like miRNA theyre transcribed from own genome
② Are primarily transcribed in cells of germ line where they interact w/ germ cell protein Piwi
③ In germ line piwi and piRNAs act together to silence any transposons that have base pair sequence with the piRNA
  ↳ Silencing occurs through DNA methylation & heterochromatin formation around Transposon integration (this occurs in nuclease)
- Germline is demethylated which can activate transposons which were silenced which can cause Insertional mutagenisis
*piRNAs Keep transposons from jumping thus help prevent transposons from causing heritable mutations
- Genome comparison of P strain & M strain showed they had P element transposons and piRNAs that are complementary to mRNA transcripts of Pelement transposase gene
  ↳ M strain have no Pelement & corresponding piRNAs
* Inhibitor molecule actually piRNAs that silence Pelement locus
*P strain evolved piRNA genes after initial insertion of Pelement transposon into genomic Data

What you should know

How was RNAi discovered? (Nobel prize in2006)
studied embryos injected w/ SS RNA & DSRNA and compared level of transcription in embryo

How is using RNAi experimentally different from making a knockout or generating mutants?
Knockout genes destroy gene in genomic DNA. RNAi is Knockdown so it does not damage the gene itself. This is temperal & we can study function of gene which could have been lethal

What does Dicer do to foreign dsRNA?
Cleaves the dsRNA randomly to produce 22 Bp fragment that is now called siRNA

What does the RISC complex/Argonaut do?
using PAZ domain Argonaut, a RNASE in RISC Complex, recognizes the 3' end of guide siRNA this puts target mRNA in position so can be cleaved by Argonauts RNASe domain

What do Drosha and Dicer do to pri-miRNAs and pre-miRNAs, and how do they recognize their targets?
Drosha cleaves single phosphodiester bond on either side of the stem loop in pri-mRNA within the nucleus and creates pre-miRNA. Drosha uses placement of bulges to decide cut sites, and leaves 3' overhang of pre-miRNA. pre-miRNA is then transported to cytoplasm where it is cleaved by Dicer. It does this by recognizing 3' overhang & cutting ~22 bp away

What is the distinction among siRNAs, miRNAs, and piRNAs? What are their roles?
Si RNA → man made used to inhibit RNA & amplify RNA
miRNA → Made in animal genome & used for RNA inhibition
piRNA → Made in genome & w/ pewdie works to silence transposons in genome

RNA I
Dicer

Si RNA
Dicer (cytoplasm)
↳ Risc
↳ Argonate
↳ Paz Domain

*In SiRNA if mRNA is highly compatible will degrade it

miRNA
Drosha (in nucleus)
↳ Dicer (same as SiRNA but here cut not random)
↳ Paz Domain
↳ Ris c
↳ Argonate

has to be processed by Droshar before goes to cytoplasm

Maturation of RNA

SiRNA → Dicer cleaves foreign RNA into 22 bp pieces
Risc binds siRNA which is ds causing it to denature, denatured RNA will target RISC to sequence which it's homologous to
Mi RNA → Formed by standard euk. transcriptional machinary
Functional sequence is cleaved by 2 ribonuclease releasing stem loops
Stem loops are proccessed by Drosha to give mature miRNA

Roles of RNA in cell

mRNA → encodes protein
tRNA & rRNA → Involved in translation
SnRNAs → Involved in splicing
si RNA/miRNA → Involved in RNA regulation
Riboswitche → Involved in expression regulation
CRISPR → Involved in immunity

Dicer is RNASE III

Argonate called slicer does initial mRNA cleavage

RNA dependent RNA polymerase → amplifies inhibitory signal so generates dsRNA after recruited to mRNA by original SiRNA

SiRNA vs miRNA

SiRNA: Come from DS RNA which enters the cell from external source
Mi RNA: Encoded genes withen chromosome

Mode of action of the RNA

Si RNA → has 2 modes which both involve RISC removing RNA from equation, this occurs through silencing or degredation of target RNA

miRNA → works same way binds to RISC leading to silencing or degredation. But genetically encoded miRNA is specific to certain mRNA

Where does interfence occur & Synthesis

Interference → Always cytoplasm
Synthesis → miRNA (nucleus)
Si RNA (cytoplasm)

SiRNA described to work in cis since they are generated by transcripts of the regions on which they act
*Dicer is only RNA cleaving enzyme needed for SiRNA
miRNA made from splicing of pri-miRNA
↳ First cleave liberates stem loop (pre-miRNA)
*Drosha & Dicer needed but Drosha miRNA Specific

PiRNA not generated by dicer but bind to Argonaut in RISC complex