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RNA Silencing
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7.90 / 6.874 Lecture

siRNA

Exogenously introduced dsRNA, processed by dicer into siRNAs (small interfering 22-mer RNAs)
siRNAs complex with RISC (RNA induced silencing complex), bind to mRNA
-> break the mRNA
Happening in the cytoplasm
RIST (nuclear version of RISC)

siRNAs that silence DNA

H3K9 is methylated in this reaction (found in *S. pombe*)

Exists (siRNA) ncrRNA that is responsible for silencing DNA

lin-4 (1993) hairpin loop (miRNAs)

Targets lin-14 or lin-28 3' UTR mRNA

miRNA genomics in animals

22-mer
Hairpin precursor
Dicer / Drosha processed (Drosha recognizes hairpin)
ncRNA genes (e.g. mir-1) -- mostly Pol II transcription
Some miRNAs transcribed as a cluster (e.g. miR-35 - miR41)
Expression profile of miRNAs in 24 human tissues
(within 50 KB - they are co-expressed, probably from the same transcript)
Some are expressed from introns (correlated with expression of host gene)
 10^3 -> $.5 \times 10^5$ miRNA copies / cell
One miRNA can target 20 mRNAs / hour in vitro
When miRNAs do not target for degradation, may need more miRNAs as
pairing is 1-miRNA to 1-mRNA
MiRscan to find hairpins outside of ORFs
In *C. elegans* 110 - 120 found miRNA genes
In mammals number unknown
1 - 2% of human genes are thought to be making miRNAs

miRNAs and their functions in plants

20 conserved families
83 unique targets
63 transcription factors (only 6% of genes are TFs in plants)
5 F-box genes (protein degradation)
2 genes of miRNA (dicer - DCL1, and RISC complex AGO1)
Total ~70 with roles in plant development
8 - 21 non-conserved families
Conservation between miRNA and message portion of mRNA often key because -
Plant miRNAs often direct mRNA cleavage
PHABULOSA gene needed for proper leaf development
Targeted by miR165
Found that miR165 regulation is source of mutant phenotype in PHABULOSA
When miRNA can not bind -> get improper leaf development
Thesis -> asymmetric division causes miRNA targeting of existing mRNAs
for TFs as well as new TFs
Working with high-resolution in-situs to explore this question

miRNA functions in animals

HOX A, B, C, D Clusters of TF genes

Also include mir genes

miR-196 complementary to HOXB8 UTR

siRNAs - lots of RISC/RNA complexes per precursor

miRNA - one transcript -> one miRNA

Finding miRNAs

Find UTRs

Find conserved seed matches to nt 2 - 7 of miRNA

Also find all other 6-mers with equal abundance in UTRs and see how they are conserved (conserved 2.4x less) vs. other seeds

Look for seeds that are conserved across multiple UTRs

Predict candidate miRNAs based on seeds

See if candidates match UTRs

Can also use 8-mers

Targeting outside of 3' UTRs

1000 ORF targets predicted

Can also sort out functions of miRNAs

Could be more targeting, could be more miRNAs

Possible part of the targeting is non-specific and may be tolerated by non-specifically targets mRNAs