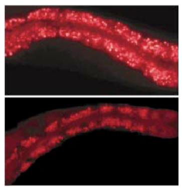
Caenorhabditis elegans



RNAi and post genomics

RNAi

Mechanism

Evolutionary origins

Applications

siRNA in mammals

Gene knockdown vs Gene knockout

RNAi mediated gene knockdown

Double stranded RNA can be injected into the gonad or the gut

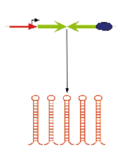
Alternatively worms can be soaked in dsRNA solution or
fed dsRNA expressing bacteria

Phenotype expressed by F1 progeny

RNAi can be applied to several genes simultaneously

RNAi mediated gene knockdown

RNAi is less effective in some tissues on later acting genes



Transgenes that generate message coding for inverted repeat sequences can circumvent this problem

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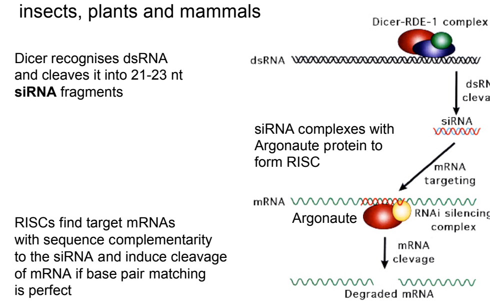
RNAi mediated gene knockdown

RNAi exploits a highly conserved mechanism in nematodes, insects, plants and mammals

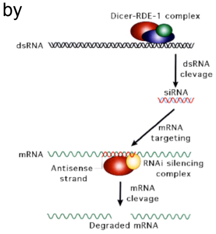


RNAi mediated gene knockdown

Double stranded RNA is produced by retroviruses and retrotransposons during their replication

RNAi thought to have evolved as a defense.

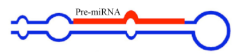
Mutations affecting the RNA cleaving domain of Argonaute make worms susceptible to retroviruses



miRNAs and gene regulation

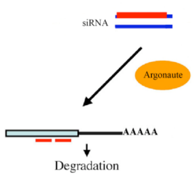
Lin-4 and *let-7* mutations delay the transition from L1-L2 and L4-adult

Neither codes for a protein but both give rise to a 60-70 nt RNA product containing hairpin-like secondary structures



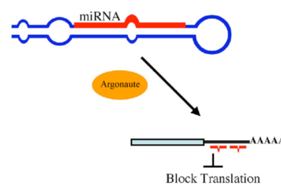
Pre-miRNAs are processed by Dicer to generate double-stranded miRNAs 21-23 nucleotides long

miRNAs and gene regulation



Unlike siRNAs, miRNAs do not <u>exactly</u> match any target mRNA sequences

miRNAs and gene regulation

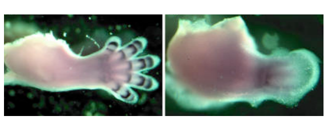


miRNAs bind to multiple complementary sequences in the 3' untranslated region of target mRNAs to block translation

miRNAs and gene regulation

The *C. elegans* genome contains 100-300 miRNA encoding genes

The human genome contains ~1000 miRNA genes many expressed only in specific cell types



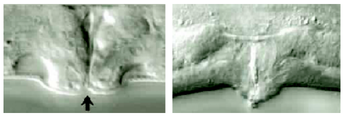
RNAi applications

1) Determining the phenotype of genes identified by sequence homology or in vitro function

2) Genome wide screening

RNAi applications

Mutations in lin-11 cause defects in vulva morphogenesis



+/+ *Lin-11/lin-11*

Lin-11 expressed in primary and secondary vulval cells

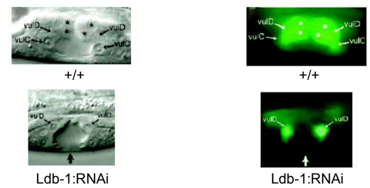
Lin-11 codes for a LIM homeobox protein

RNAi applications

Ldb-1 codes from a LIM binding protein

Ldb-1 is expressed in a subset of *lin-11* positive cells

RNAi for *Ldb-1* causes subtle defects in the expression of vulval cell differentiation markers



RNAi and post-genomics

Complete *C. elegans* genome sequence available since 1998

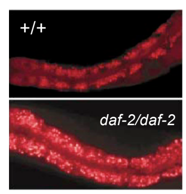
E. Coli RNAi libraries now cover 86% of identified genes

Genome-wide RNAi screens performed for genes that regulate fat storage/mobilisation, longevity and genome stability

C. elegans obesity models

Lacks dedicated fat storage cells

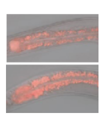
No identifiable leptin homologues



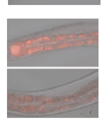
Mutations in insulin signaling genes and serotonin have similar effects on fat storage in worms and mammals

C. elegans obesity models

112 gene knockdowns cause increased fat storage



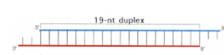
305 gene knockdowns cause decreased fat storage



Over 50% of these genes have mammalian homologues not previously implicated in weight regulation

Mammalian RNAi

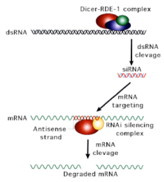
The mammalian response to dsRNA is to induce programmed cell death by activating α interferon



siRNA does not activate α interferon but still induces degradation of specific mRNAs through RISC action

Introduction of synthetic siRNA or transgenes coding for short inverted repeat sequences induces effective RNAi in human and mouse cells

Pros and cons of siRNA gene knockdown



Quick and inexpensive

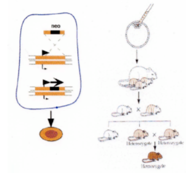
Simultaneous knockdown of multiple genes

Tissue and stage-specific

Non-specific effects

Incomplete knockdown

Pros and cons of gene knockout via ES cells



Tissue and stage-specific

Effects of point mutations

Effects very specific to gene

Knockouts complete

Time consuming and expensive

Simultaneous knockdown requires interbreeding

Summary

RNAi is a powerful technology applicable in many different systems

Understanding its mechanism has lead to new insights into gene regulation

References

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