RNAi - A silence that speaks volumes

Pawan Dhar
SBG@BII
Glossary

• Sense and antisense RNA
• Cosuppression: TGS or PTGS
• TGS: Gene Specific methylation
• RISC: RNA Induced Silencing Complex
• RNA interference
• siRNA
Transcription

5' ...ATGGAGTCTTGACATCA... 3'
3' ...TACCTGARAGT... 5'

Sense strand of DNA
Antisense strand of DNA

Transcription of antisense strand

5' ...AUGGCUUGGACUUC... 3'
mRNA

Translation of mRNA

Met — Ala — Trp — Thr — Ser — Peptide
1. Ribosome cannot gain access to dsRNA
2. dsRNA is quickly degraded
Application of Antisense technology

Flavr Savr tomatoes

Transgenic varieties
10% normal enzyme
(involved in ripening)
The Petunia Story, 1990
Author: Rich Jorgensen

- Deep Purple gene transfer
- Expected: Deepened purple color
- Observation: Variegated / white color?
- Termed it Cosuppression
The First Evidence
dsRNA - Gene Silencer

- Organism: C. elegans
- Gene par - 1
- Antisense RNA induced GS
- Sense RNA induced GS !!!
- dsRNA induced Gene Silencing
C. elegans experiment solves the puzzle ... by accident

- Inject dsRNA mix (sense & antisense)
DNA Transfer with a Gene Gun

Drosophila
Biochemical Mechanism

• Identified ~25 nucleotide RNAs (plants)
  Complementary to both sense and antisense RNAs

• Identified ~21-23 nt pairs in Drosophila
  Complementary to both sense and antisense RNAs
How does RNAi work - 1

- First step: Initiation
dsRNA is digested into 21-23 siRNAs

  Enzyme: Dicer (Member of RNase III family)

May be supported by
C. elegans genes
rde-1, rde-4
How does RNAi work - 2

- Second step: Effector

  siRNAs bind to RISC (RNA Inducing Silencing Complex)

- RISC (inactive) ---> RISC (Active)
  ATP dependent, unwinding of RISC

May be supported by
C.elegans genes rde-2, mut-7
How does RNAi work - 3

• Active RISC
• Targets homologous transcripts
• Cleaves them 12 nt from 3’end of siRNA
• Mechanism of cleavage: unclear
• RISC composition: single siRNA, RNase
• Amplification step: 
  *dsRNAs or siRNAs*?
Genes that promote PTGS

• Neurospora, qde-1
• Arabidopsis SDE-1/SGS-2
• C.elegans, ego-1

• May suggest RNA-dependent RNA polymerases (RdRPs) linked to RNAi
Recap: RNAi
Another view: RNAi
In-situ hybridization showing RNAi effect

C.elegans
RNAi overview

- Watchdog of the cell
- Seek-n-destroy tool
- dsRNA (Exogenous)
- Aberrant RNA
PTGS - The silk route

- **Strong evolutionary basis** (C.elegans, Drosophila, trypanosomes, zebra fish, mice, human)
- **May have evolved as defense mechanism**
- **Disruption of RNAi supporting genes causes developmental defects !!**

stRNA STORY - NEXT
Small Temporal RNAs (stRNAs)

- Regulates C. elegans development

- Lin-4 and Lin-7 stRNAs generated from 70 nt long transcripts folded into stem loop structure

Cleaved by Dicer !!
Application

- Non-specific gene silencing by long dsRNAs
- Specific gene silencing by siRNAs
- Potential tool for functional genomics
- ~19,000 genes in C. elegans
- Gene therapeutics
Limitations

• Silencing effect temporary

• Uptake by cells merits optimization
References

- http://www.ambion.com/hottopics/rnai
- Nature 2002: 418, 244 - 251
- Nature 2000: 404, 804-808
Animation

URL:

http://www.nature.com/nrg/journal/v2/n2/animation/nrg0201_110a_swf_MEDIA1.html