RNA interference (RNAi)

Natasha Caplen

Gene Silencing Section

Office of Science and Technology Partnerships

Office of the Director

Center for Cancer Research

National Cancer Institute

ncaplen@mail.nih.gov

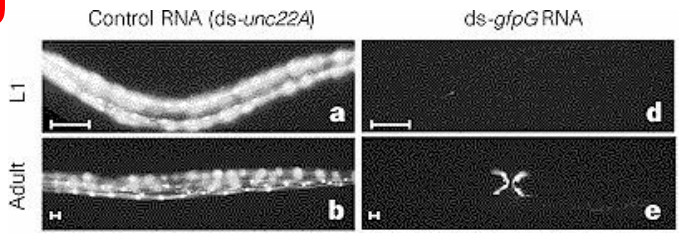
OSTP Office of Science & Technology Partnerships

RNA silencing

Plants

Napoli et al., Plant Cell (1990) 2 289
Van der Krol et al., Plant Cell (1990) 2 291

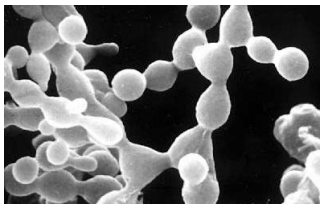
C. elegans

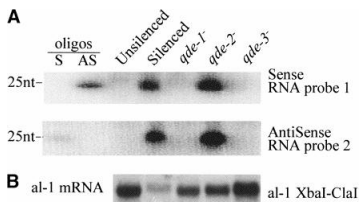


Fire *et al.*, Nature (1998) 391 806

Double stranded RNA

Fungi



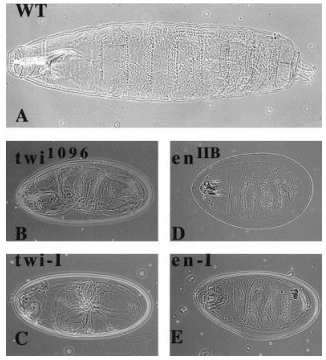


Romano & Macino Mol. Microbiol (1992) 6 3343

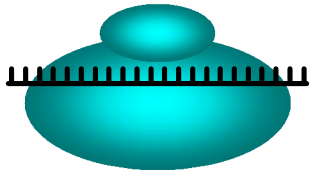
dsRNA processing

Small interfering RNAs (SiRNAs)

Drosophila

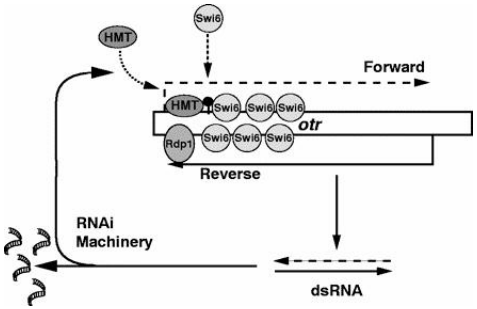


Misquitta & Paterson PNAS (1999) 96 1451
Kennerdell & Carthew Cell (1998) 95 1017



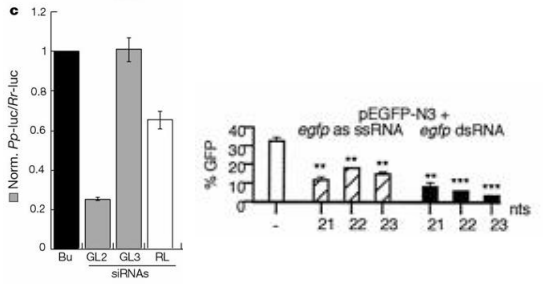
RNA induced silencing complex RISC

S. pombe



Volpe et al., Science (2003) 292 1833

Mammals



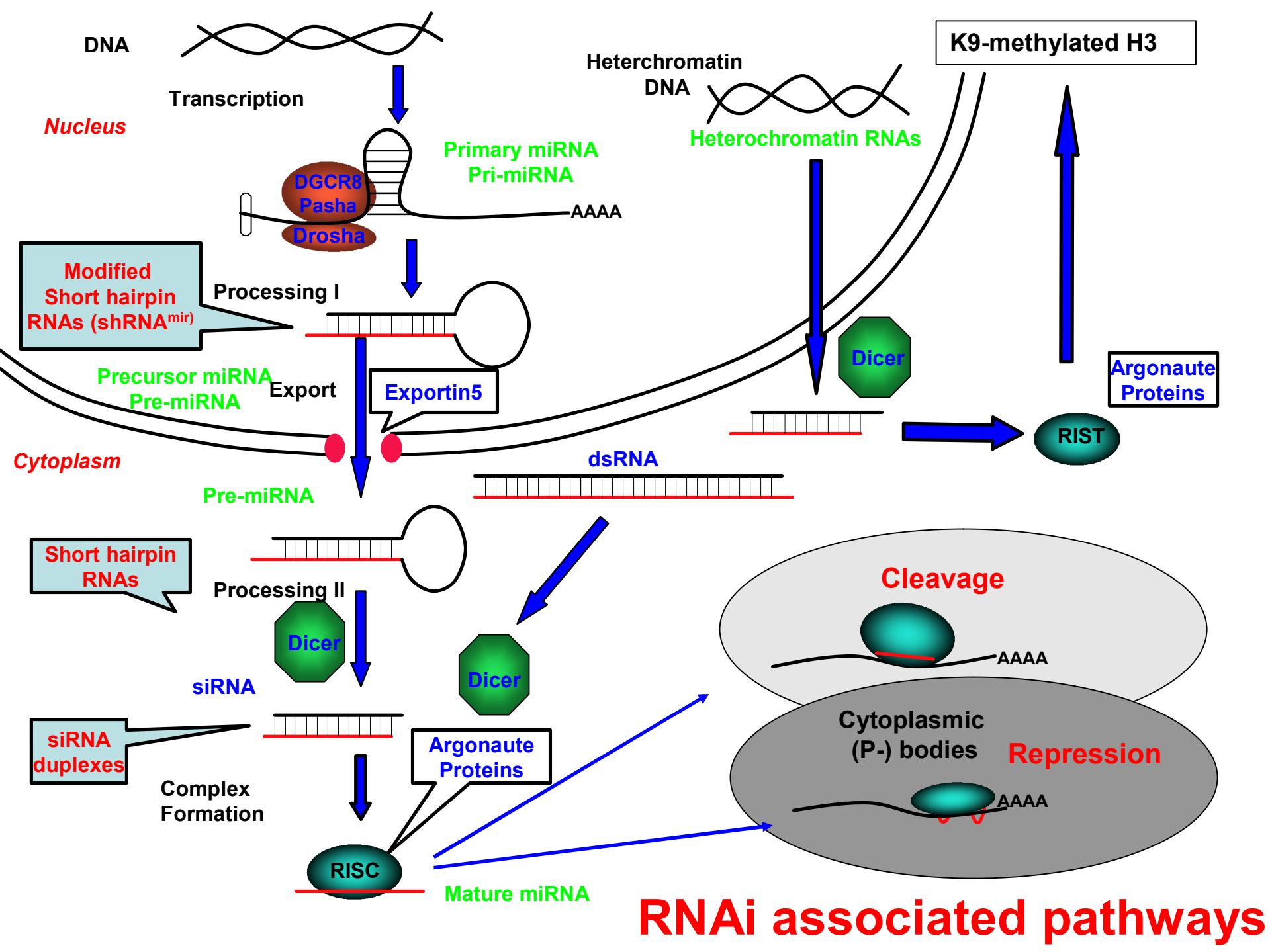
Elbashir *et al.*, Nature (2001) 411 494
Caplen *et al.*, PNAS (2001) 98 9742

RNA interference (RNAi) & its associated pathways

- Endogenous mechanism(s) controlling
- gene expression via silencing
- Requires double stranded RNA (dsRNA) or
- dsRNA domain-containing molecules
- Associated with a number of conserved proteins containing dsRNA binding domains
- Biological roles include:
  - Control of endogenous gene expression
  - from protein encoding mRNAs
  - Heterochromatin formation
  - Silencing of “selfish” genetic material
  - Antiviral response

RNAi

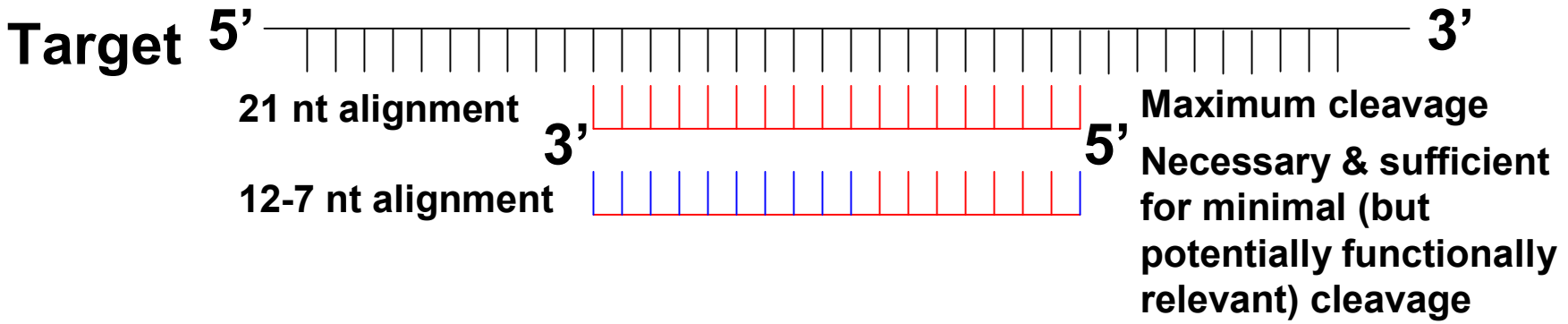
- **Down regulation mediated by RNAi can occur at a:**
  - **DNA, RNA or protein level**
- **Determined by:**
  - **Characteristics of the dsRNA trigger**
  - **The proteins incorporated with the RNAi mediating RNA-protein complex**
  - **The interaction of the small RNA molecule and the target sequence**


DNA
Transcription
Nucleus
Primary miRNA
Pri-miRNA
DGCR8
Pasha
Drosha
AAAA
Modified
Short hairpin
RNAs (shRNA^mir)
Processing I
Precursor miRNA
Pre-miRNA
Export
Exportin5
Cytoplasm
Pre-miRNA
Short hairpin
RNAs
Processing II
Dicer
siRNA
siRNA
duplexes
Complex
Formation
Argonaute
Proteins
RISC
Mature miRNA
Heterchromatin
DNA
Heterochromatin RNAs
K9-methylated H3
Dicer
Argonaute
Proteins
RIST
dsRNA
Dicer
Cleavage
AAAA
Cytoplasmic
(P-) bodies
Repression
AAAA
RNAi associated pathways

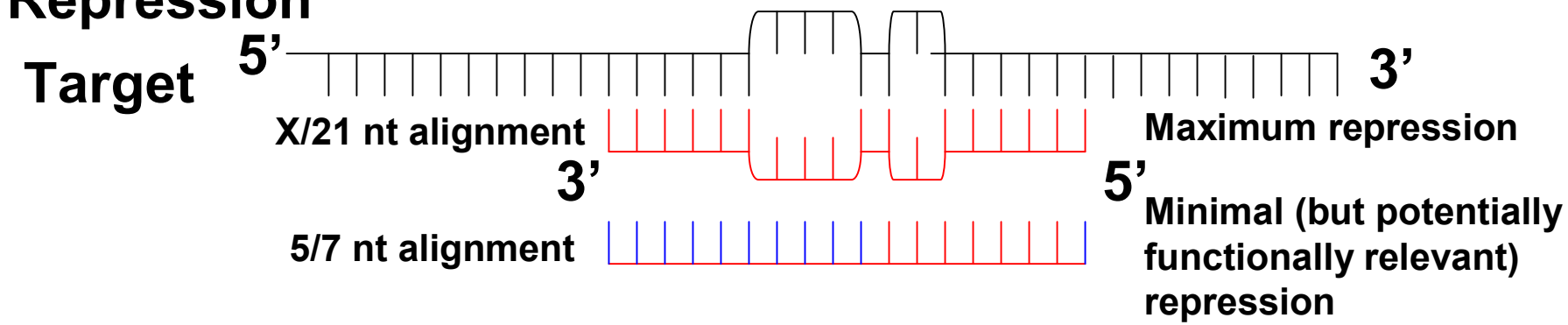
RNAi via transcript cleavage or protein blockade

Sequence alignment is of importance in determining the mode (cleavage versus repression) and degree (efficacy of cleavage and/or repression)

Cleavage



Repression



Endogenous triggers of RNAI: MicroRNAs

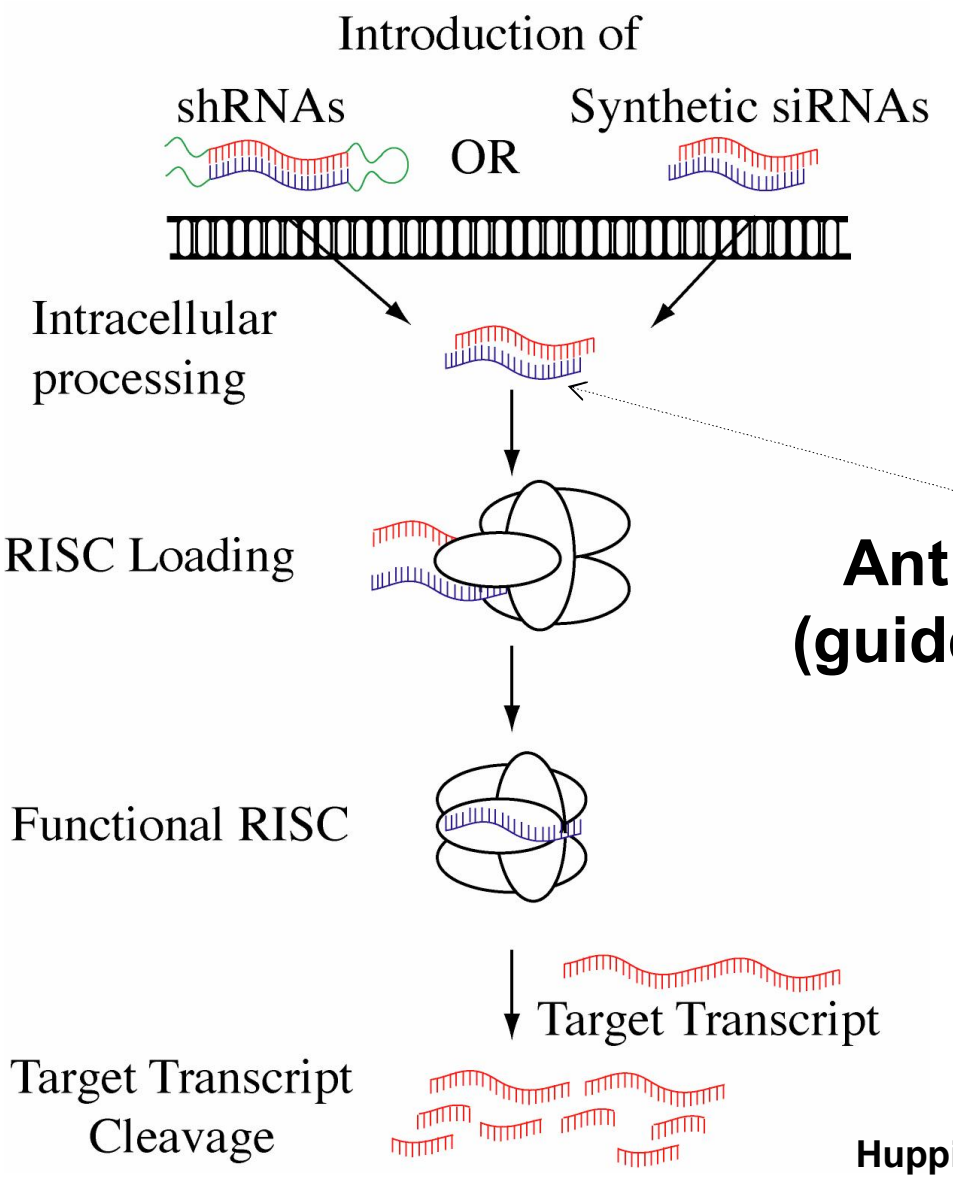
- A naturally occurring small species of ssRNA, ~22 (17-24) nts in size
- Precursor and mature miRNAs interact with many of the same proteins as exogenous small RNAs (siRNAs) that mediate RNAi
- Interaction with target mRNA decreases protein levels

  miRNA - RNA induced silencing complex (RISC) interaction with a transcript may induce

  - (a) Translational repression
  - (b) RNA cleavage

- Epigenetic regulation of gene expression through miRNA: transcript interactions appears to be critical for multiple cellular process including essential roles in development and differentiation

Exogenous triggers of RNAs: shRNAs and siRNAs



Antisense (guide) strand

Huppi, Martin, Caplen
Molecular Cell (2005) 17 1-10

Two principal RNAi effectors
Synthetic siRNAs and short hairpin RNAs

Synthetic siRNAs

- Double strand oligonucleotides
- Sense r(N19)dTdT,
- antisense r(N19)dXdY

- **Short hairpin RNAs**
  - Single strand molecule requiring
  - intracellular processing
  - Can be expressed intracelluarly

Applications of RNAi

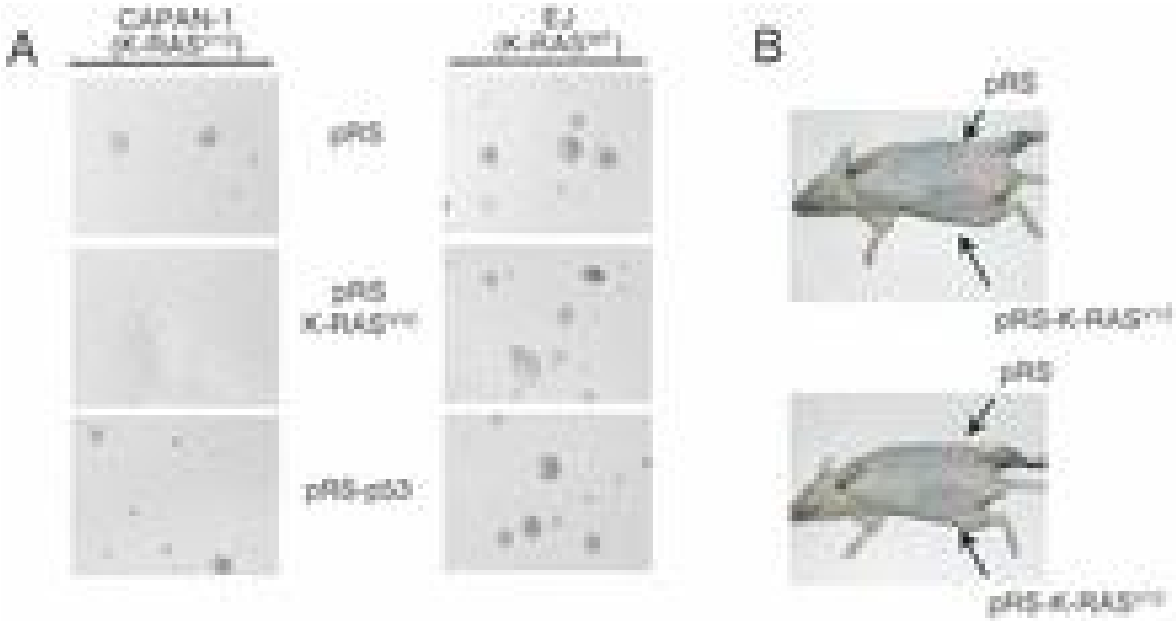
- **Functional genomic analysis**
  - **up to whole genome**
- **Drug: target characterization**
- **Novel cellular model systems**
- **Transgenic animals**
- **Experimental studies of RNAi as a therapeutic**

Therapeutic potential of RNAi: Cancer

Brummelkamp *et al*., Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer Cell (2002) 2 243-247

RAS genes are frequently mutated in human cancers, e.g. 85% of pancreatic cancers, 40% or colon carcinomas harbor mutations. Ras genes are guanine nucleotide binding proteins and act as intracellular a signaling proteins and are required for regulation of cell proliferation, differentiation and survival. Therapeutic intervention requires specific elimination of the product from the mutant oncogenic allele

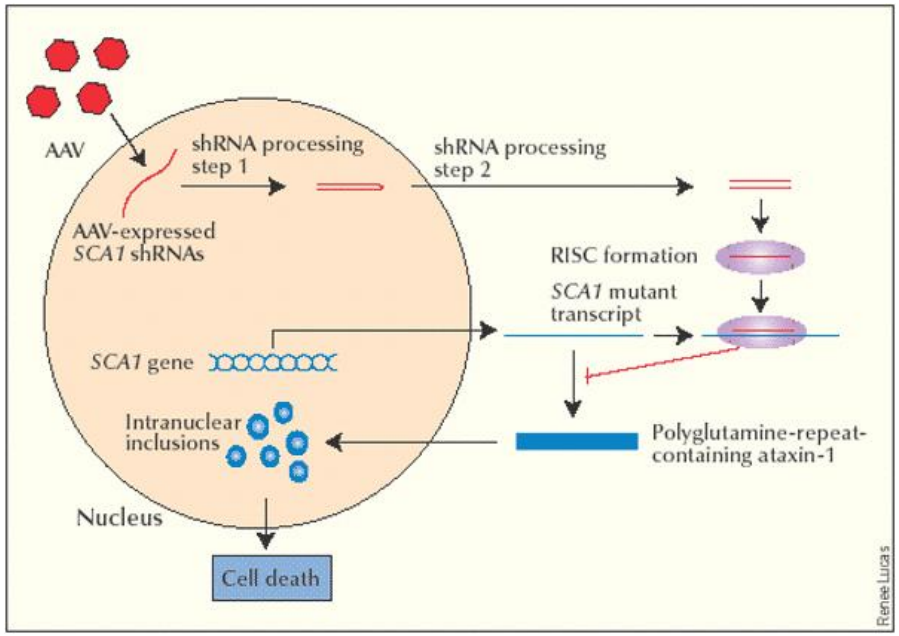
Stable & selective loss of tumorigenicity by a retroviral vector that targets the K-RASV12 oncogene

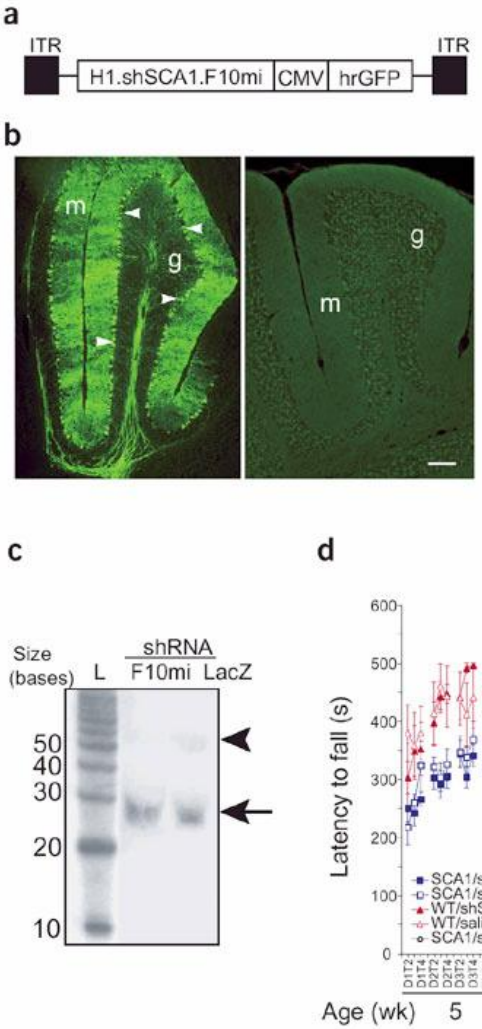


Therapeutic potential of RNAi: Dominant Genetic Disease

RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia

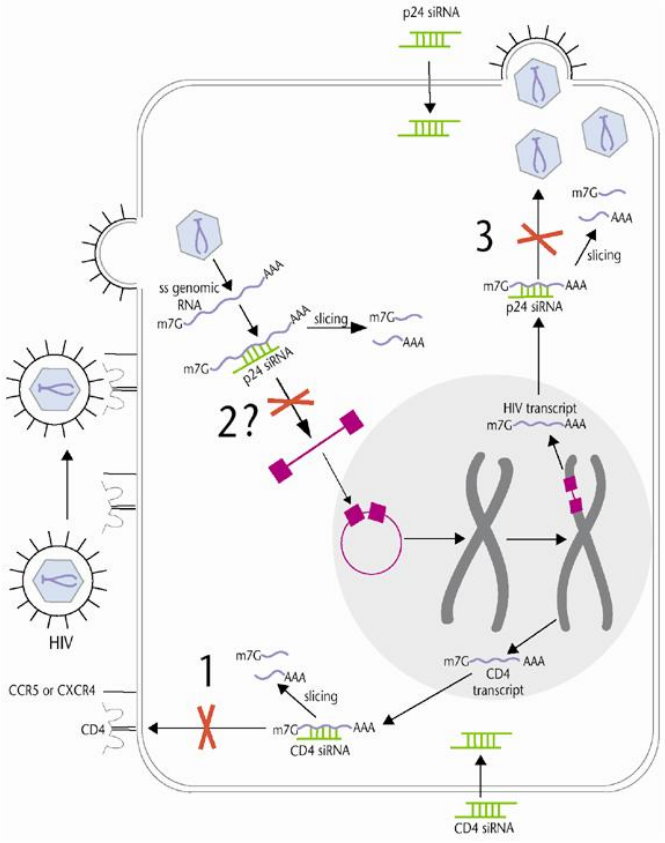
Xia et al., *Nature Medicine (2004)* **10**, 816 - 820

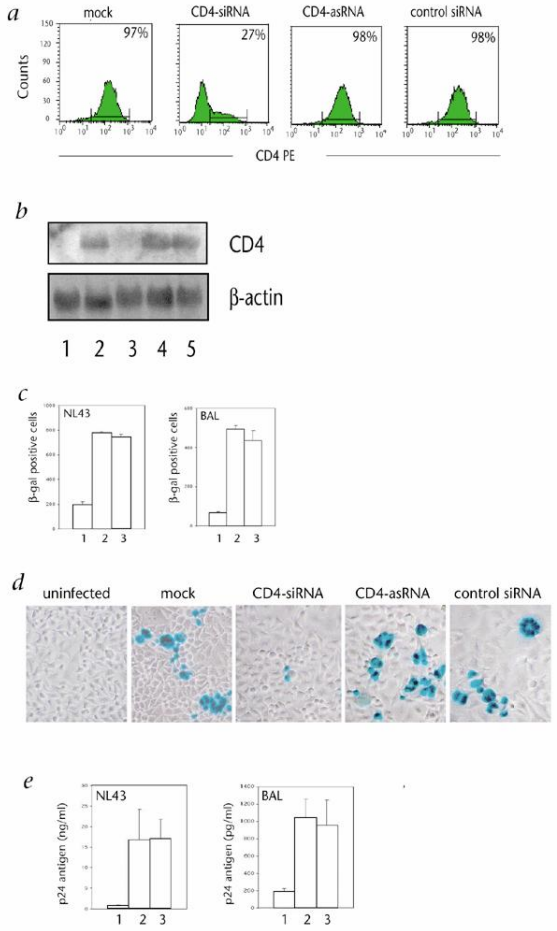




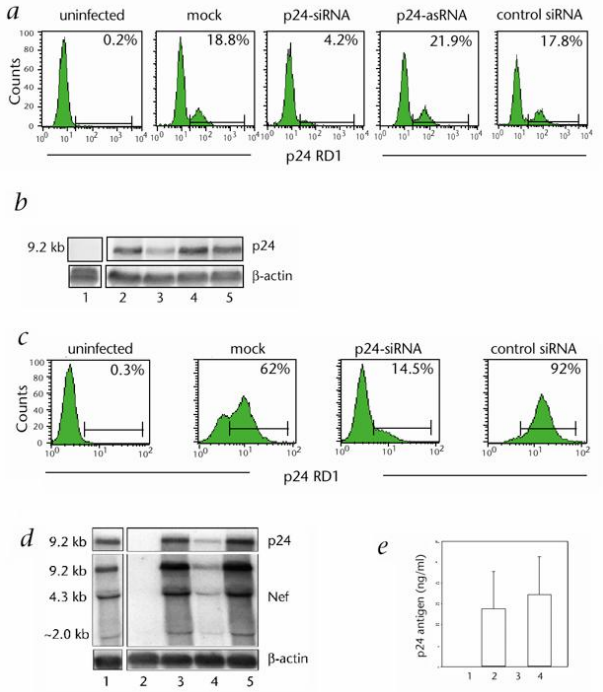
Therapeutic potential of RNAi: HIV-1 infection

Novina et al., siRNA-directed inhibition of HIV-1 infection Nature Medicine (2002) 8 681- 686.





CD4-siRNA inhibits HIV-1 entry and infection in Magi-CCR5 cells.



p24-siRNA inhibits viral replication in HeLa-CD4 cells.

RNAi and issues related to therapy

- Effects on the normal cellular role of RNAi
- RNAi effects on non-targeted transcripts
- Activation of non-specific dsRNA cellular responses
- The establishment of resistance through mutation

Effects on the normal cellular role of RNAi

Can the RNAi machinery be saturated?

- **Utilization of multiple shRNAs**
- **Transcriptome & proteome analysis**
- **miRNA expression profiling**
- **Functional analysis**

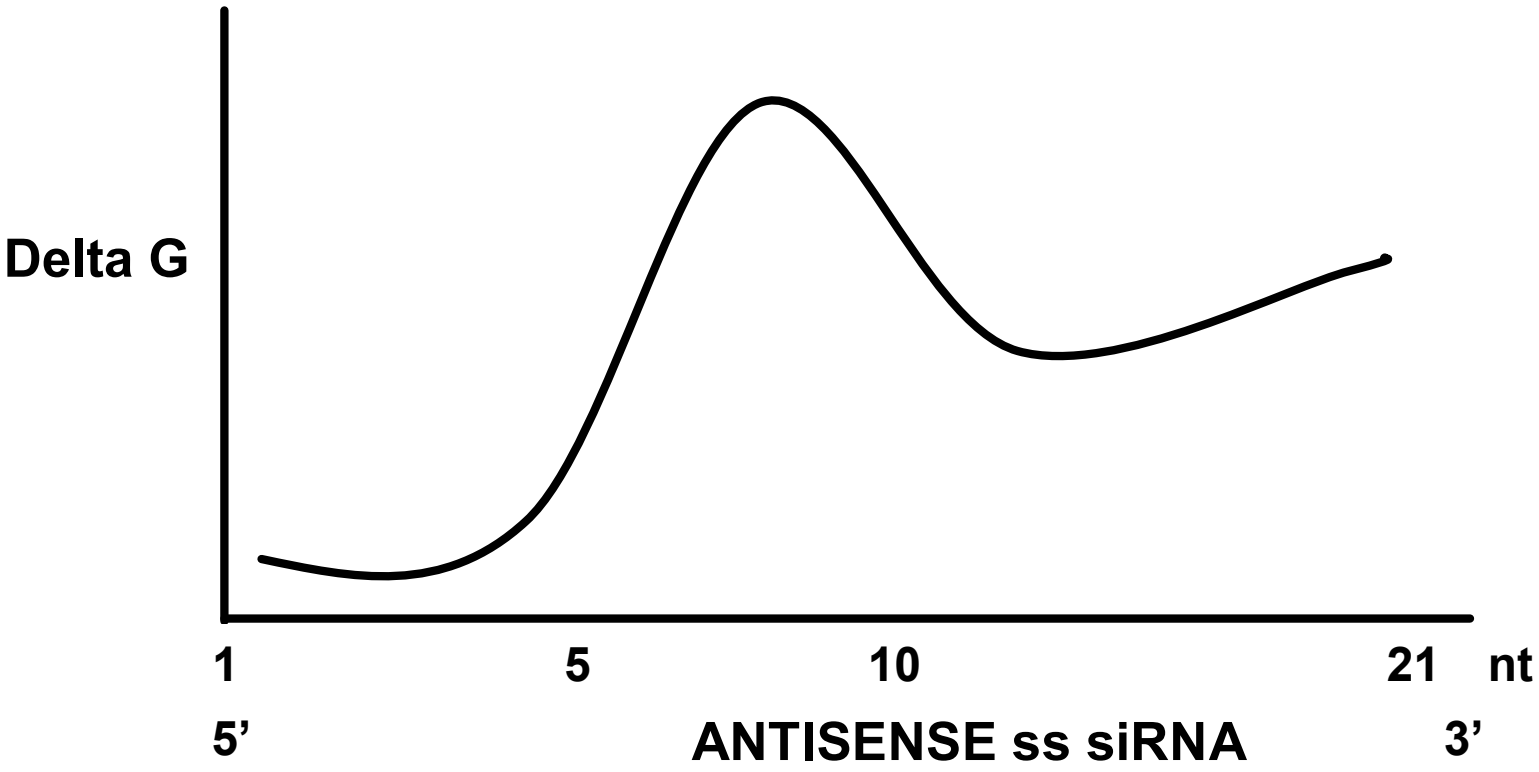
(e.g. Differentiation)

RNAi effects on non-targeted transcripts

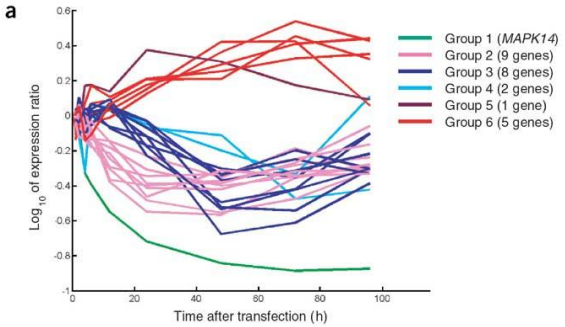
- Optimization of RNAi effector
- Microarray expression profiling
- Bioinformatics
- Functional analysis

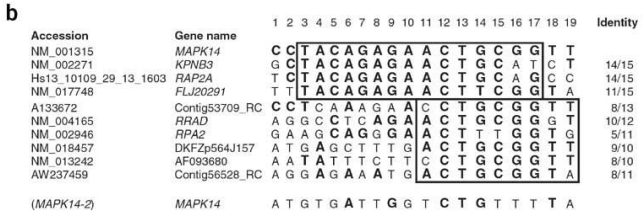
Optimization of the RNAi effector

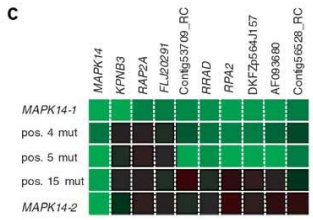
Asymmetric loading using small RNA thermodynamics can be used to favor RISC loading of the antisense (guide) strand

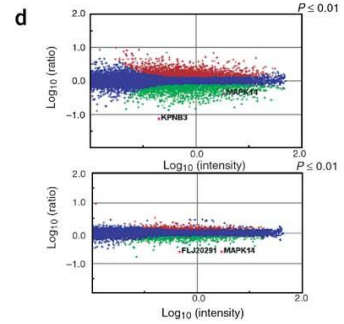


Microarray expression profiling







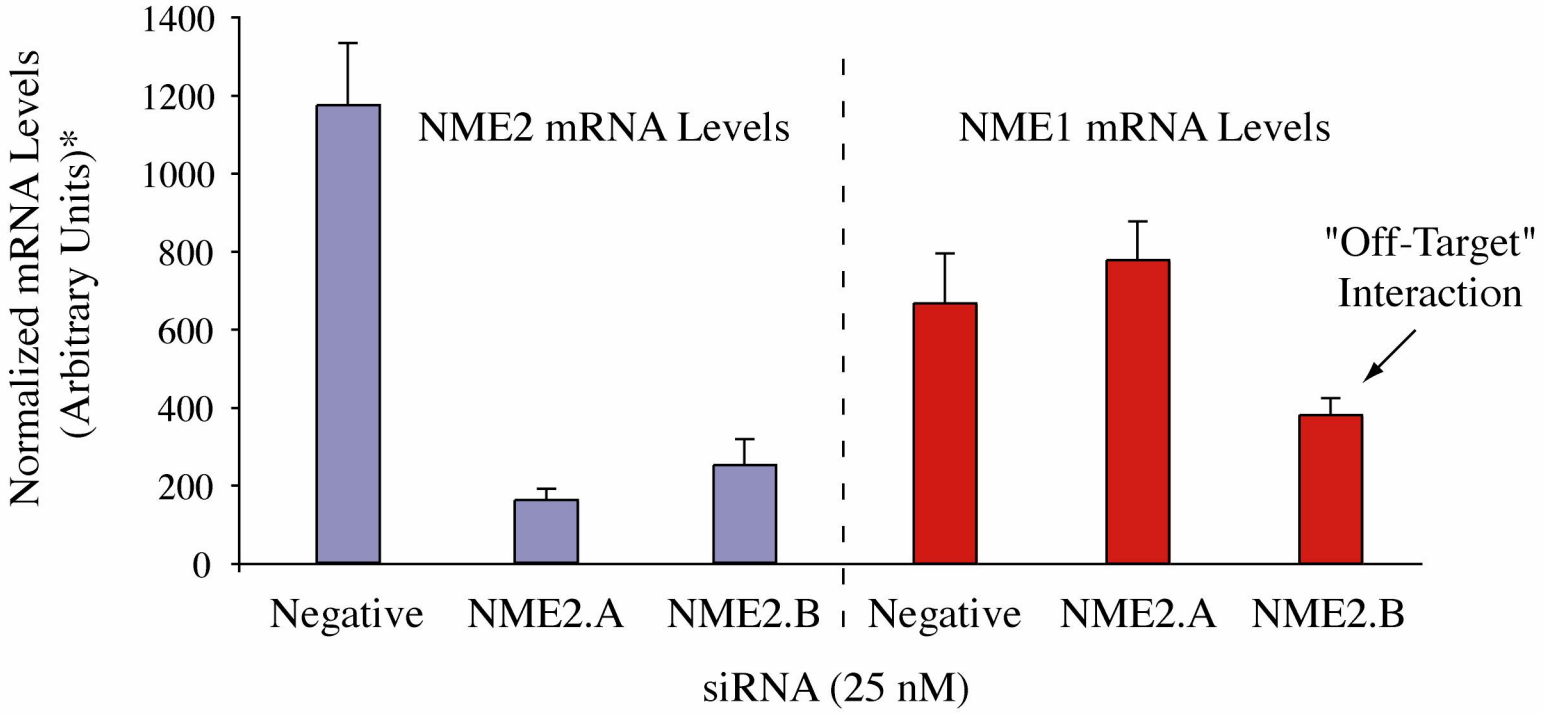


Expression profiling reveals off-target gene regulation by RNAi

Jackson *et al*., Nat. Biotech. (2003)

Multiple additional studies show that though the predominate interaction may be the targeted one there can be a significant background of ‘off-target’ interactions.

An NME2 Directed siRNA Reduces NME1 Transcript Levels



NME2 target sequence (for siRNA B): GCGTACCTTCATTGCGATCAA

NME1 homologous sequence: GCGCACCTTCATCGCCATCAA

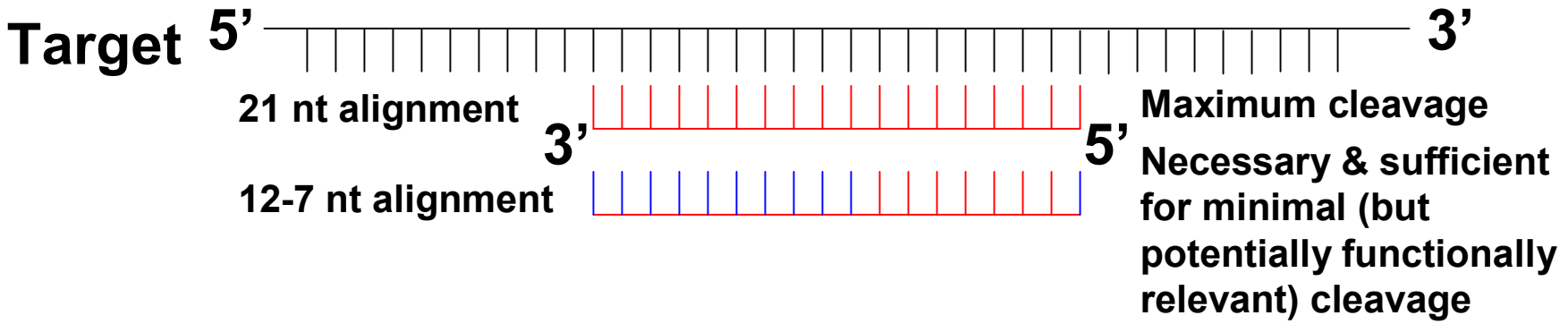
(NME1 [NM23A/H1] and NME2 [NM23B/H2] are 88% homologous at the mRNA level.

* Target transcript mRNA levels were normalized to cyclophillin mRNA levels.
Experiments performed in HCT-116 cells.

RNAi via transcript cleavage or protein blockade

Sequence alignment is of importance in determining the mode (cleavage versus repression) and degree (efficacy of cleavage and/or repression)

Cleavage



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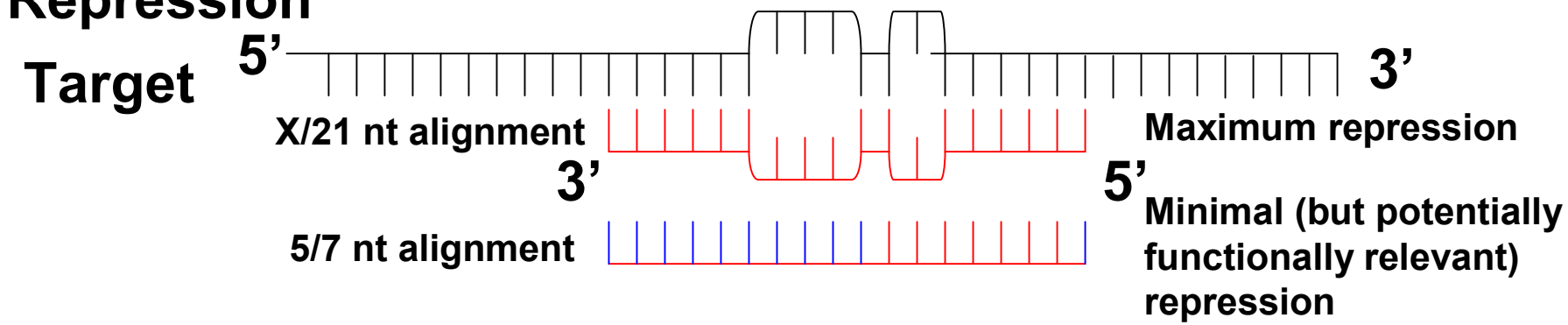
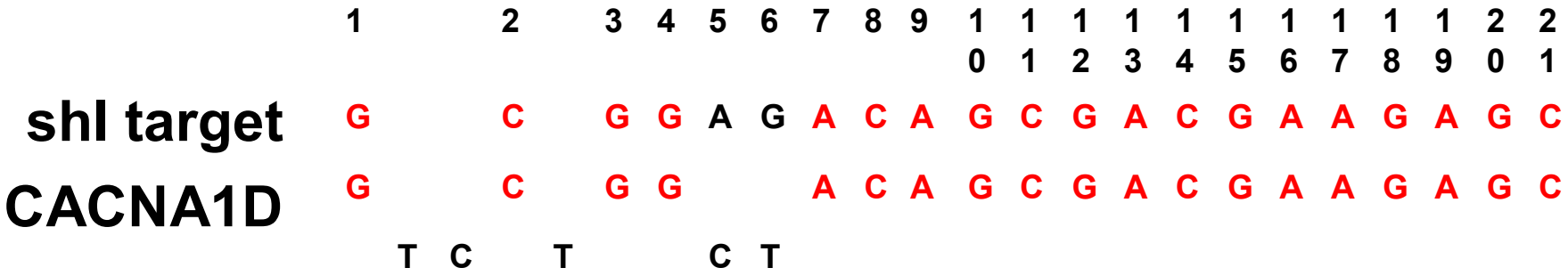


RNAi non-specific effects: Bioinformatic analysis



CACNA1D: calcium channel, voltage-dependent, L-type, alpha 1D subunit NM_000720

RNAi non-specific effects: Bioinformatic analysis

	1			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
shl target	G			C	G	G	A	G	A	C	A	G	C	G	A	C	G	A	A	G	A	G	C
TMPRSS6	G			C				G		C	A	G	C	G	A	C	G	A	A	G	A	G	C
		T	C	T		A	A	C		G													

TMPRSS6: Transmembrane protease, serine 6
NM_153609

RNAi non-specific effects: Functional analysis

Do cells remain functional intact?

RNAi and activation of non-specific dsRNA cellular responses

- **Cellular responses to dsRNA part of anti-viral response**
  - Frequently result in non-specific decrease in protein translation and cell death
  - Size and concentration may play significant role
  - Some sequence “motifs” within small dsRNAs may be more potent than others as stimulators of dsRNA cellular respones
    - Key proteins include PKR, OAS1, and Toll 3 receptor

RNAi and the establishment of resistance through mutation

- **Single nucleotide changes can eliminate RNAi**
- **Selective pressures could lead to resistance as a result of single nucleotide changes**
- **Of more signficance with respect to efficacy**

Overview

- RNAi is a potent mediate of gene silencing
- RNAi has been successful exploited as a functional genomic tool
- The move to a clinical application will be determined by the ability to assess the realistic implications of

  - any effects on the RNAi machinery,
  - any effects on other cellular transcripts and
  - minimization of non-specific effects