Joint Graduate Seminar 2009

Short Interfering RNAs (siRNAs)

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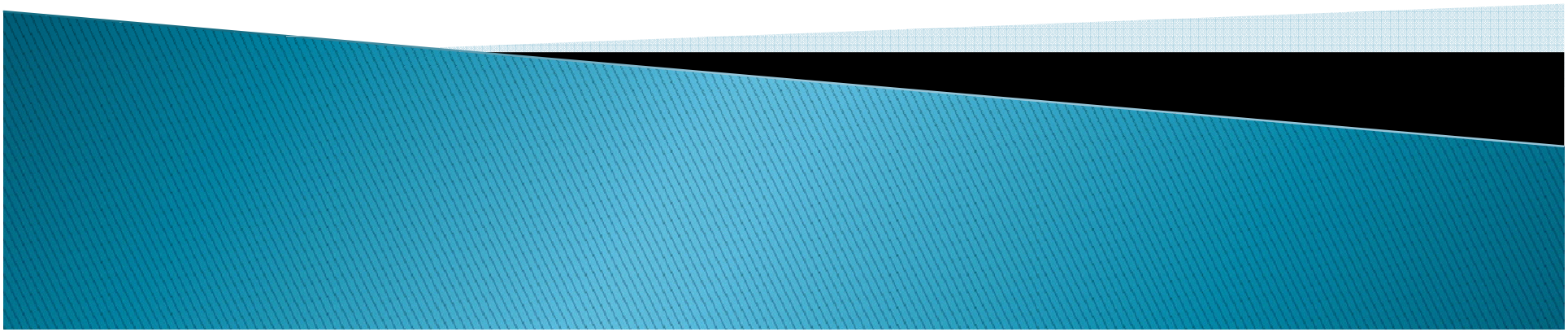
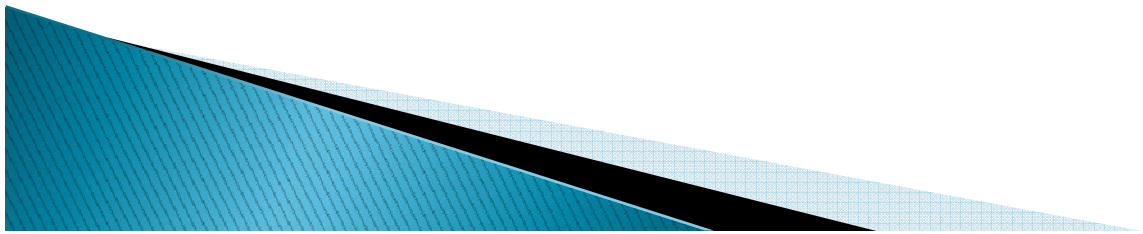


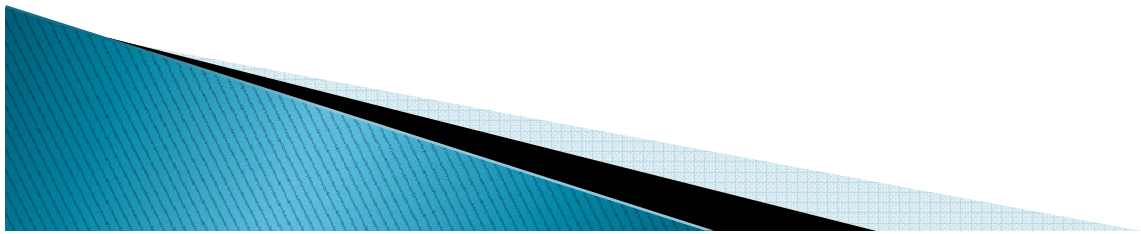
Introduction

- Non-coding RNAs
- Regulate gene expression in a sequence-specific manner
- Participate in diverse regulatory events, ranging from copy-number control in bacteria to X-chromosome inactivation in mammals



Discovery

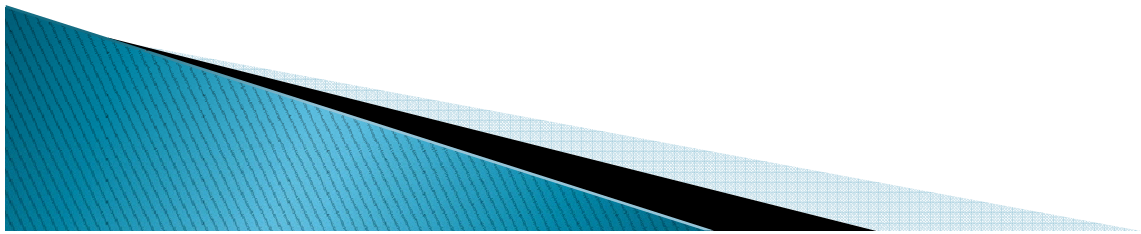
- First discovered in plant
- Function as a defence mechanism against viruses
- Plant viruses have ssRNA genomes which replicated as dsRNA during life cycle



Discovery

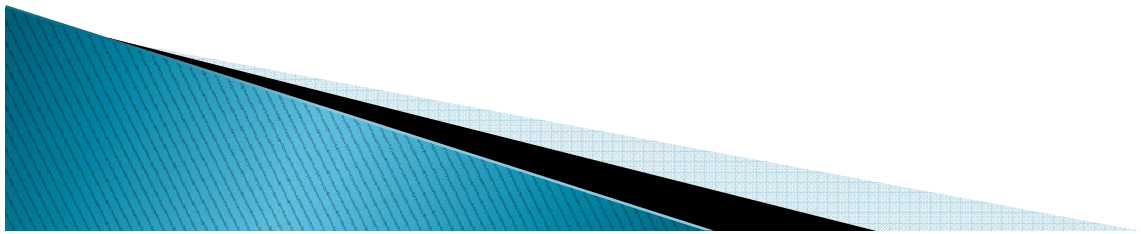
- This dsRNA triggers RNA interference (RNAi) pathway for RNA degradation

- RNAi:
  - a form of post-transcriptional gene silencing
  - dsRNA induces degradation of the homologous mRNA
  - reduction of gene expression

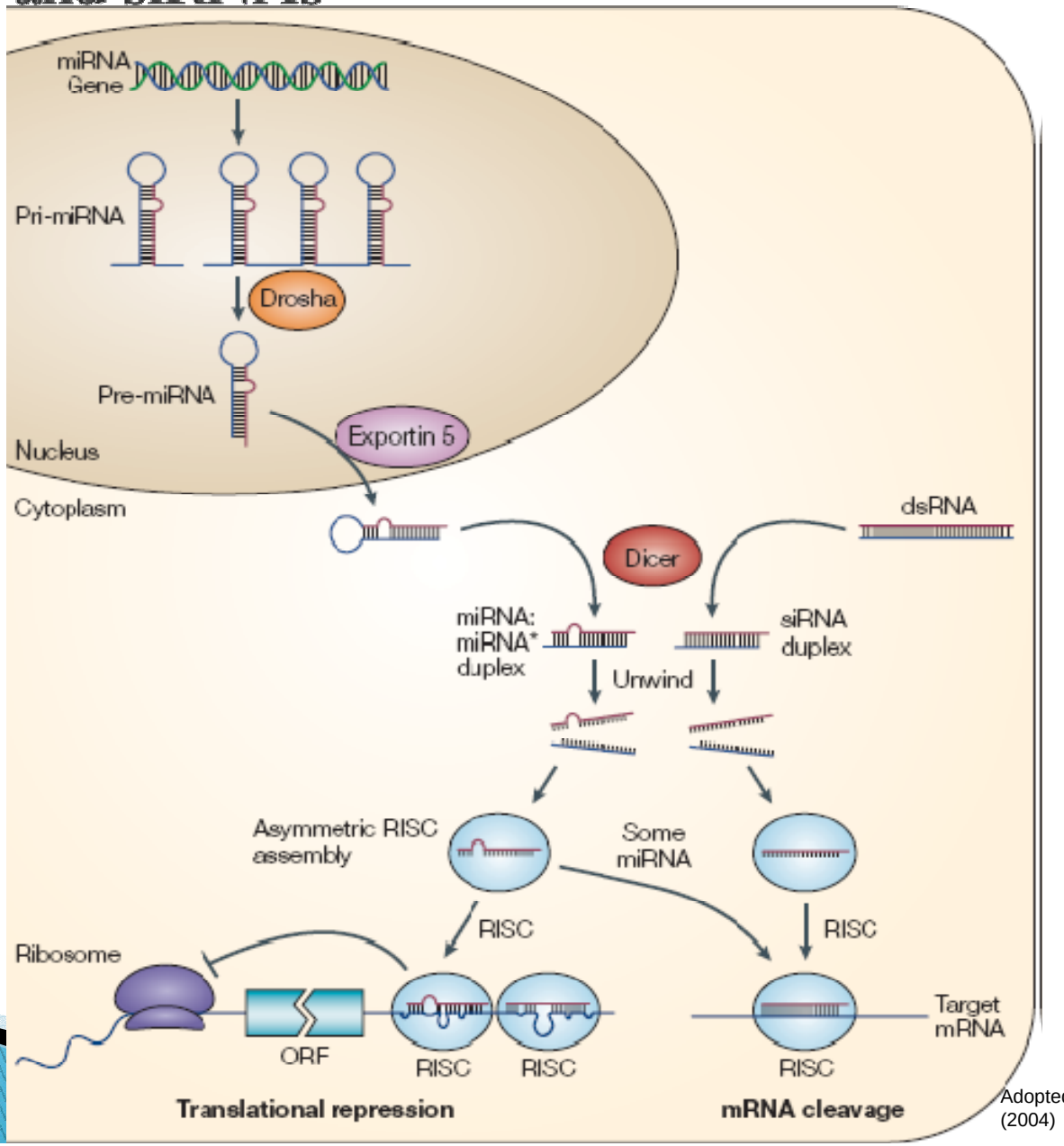


Small RNAs

- 2 classes:
  - microRNAs (miRNAs)
  - short interfering RNAs (siRNAs)
- Biochemically and functionally indistinguishable, classified based on their origin
- miRNAs: endogenous miRNA gene
- siRNAs: exogenous sources e.g. viral infection



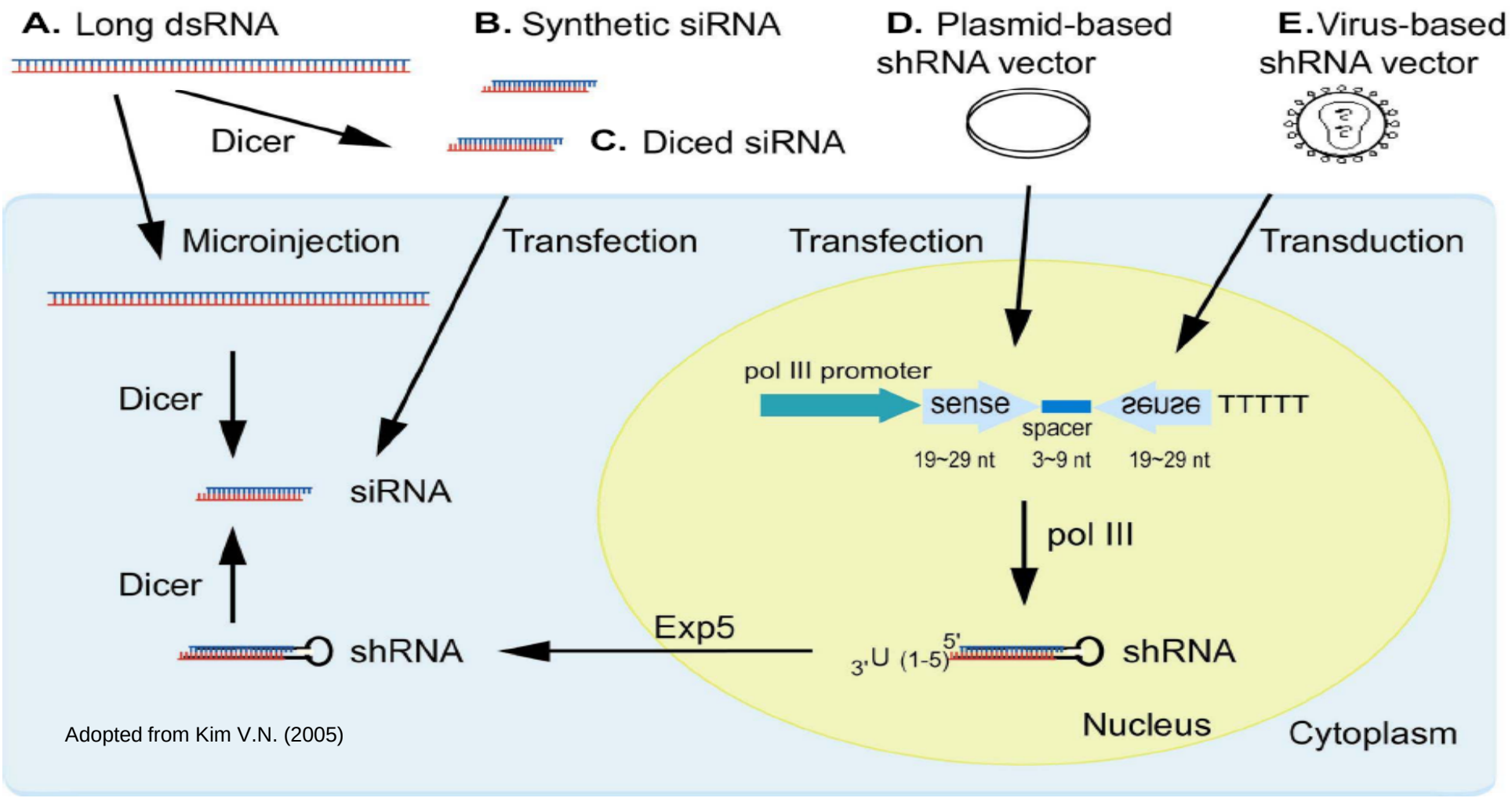
Post-transcriptional gene silencing mechanism of miRNAs and siRNAs



Adopted from He L. and Hannon G.J. (2004)

Small RNAs as an experimental tool

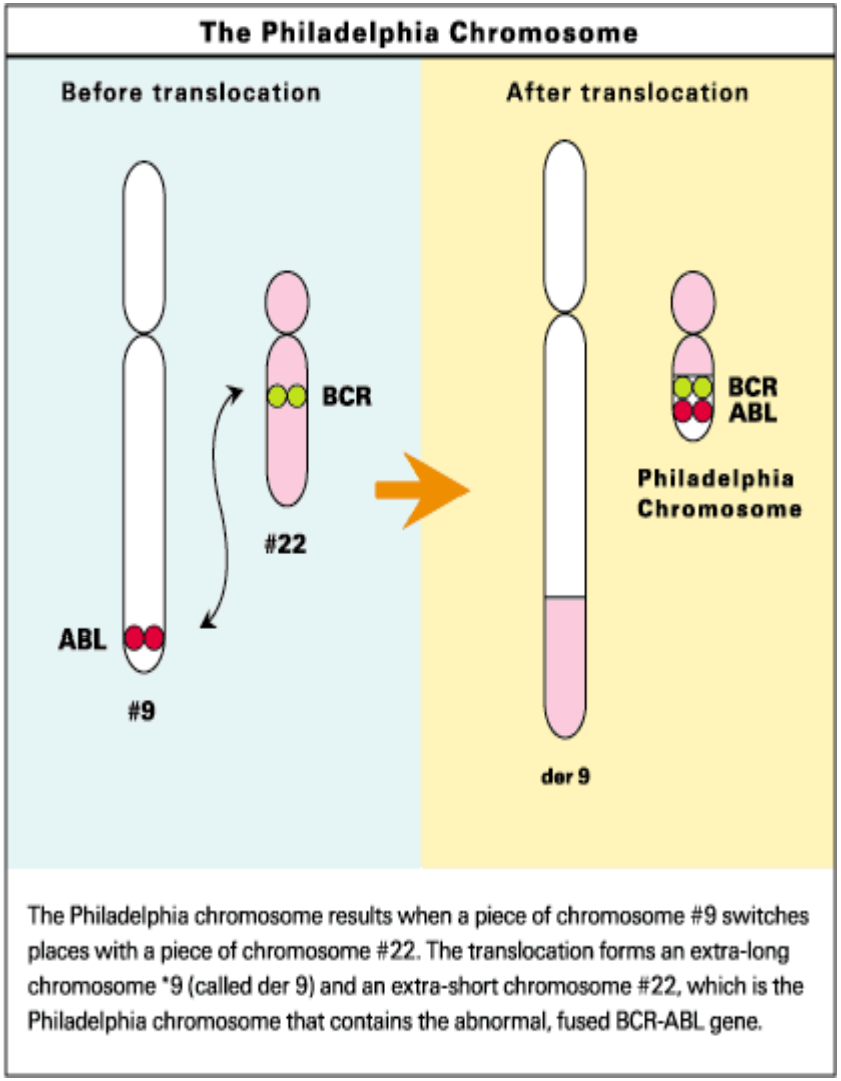
- Specificity and efficiency
- Powerful gene knockdown technique



Adopted from Kim V.N. (2005)

Therapeutic application

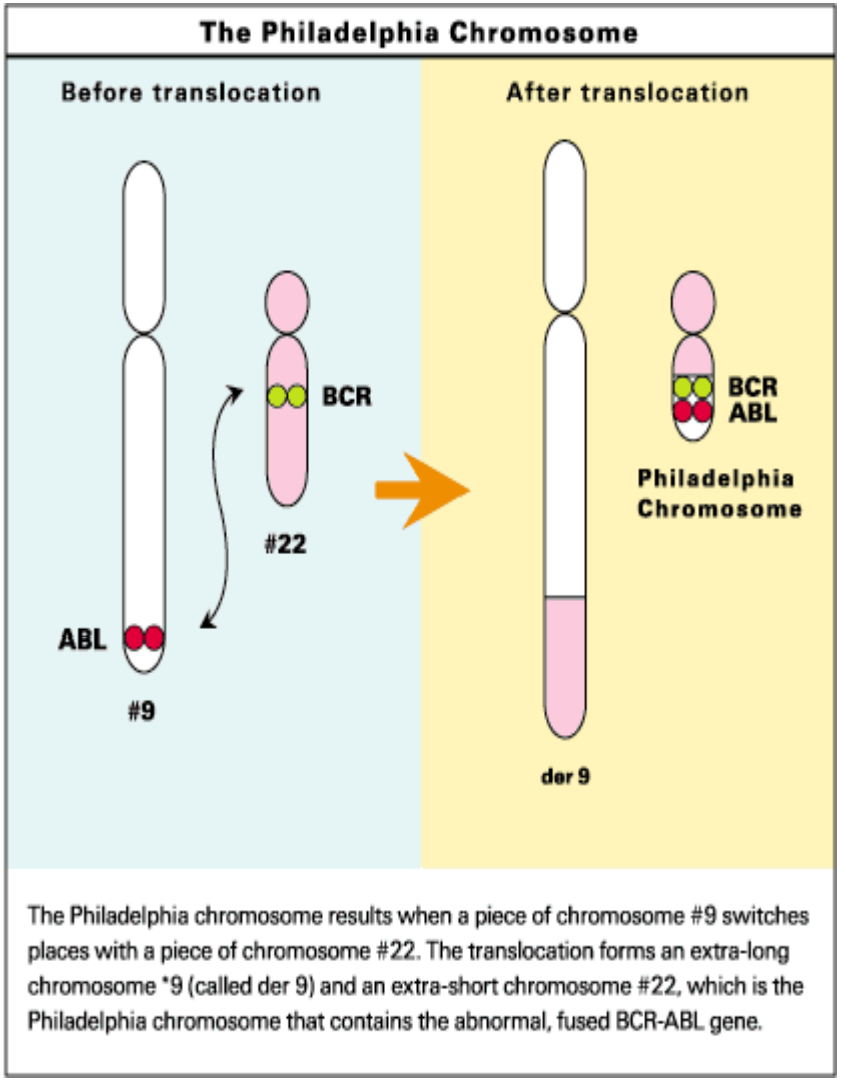
- Inhibit expression of genes involved in oncogenesis
- Philadelphia chromosome associated with leukemia
- Chromosomal translocation of Abl gene on chromosome 9 to Bcr gene on chromosome 22
- Hybrid gene for a chimeric protein
- N-terminal of Bcr protein and C-terminal of Abl protein



Adopted from Antigenics Inc. (2009)
http://www.antigenics.com/diseases/cml.html

Therapeutic application

- Abl is a protein tyrosine kinase involved in cell signaling
- Substitution of N-terminal Bcr fragment makes it hyperactive
- Stimulate inappropriate proliferation of white blood cells released into bloodstream->leukemia
- siRNAs specific for the Bcr-Abl fusion transcript have been shown to silence its expression without affecting normal Bcr and Abl gene expression levels



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Therapeutic application

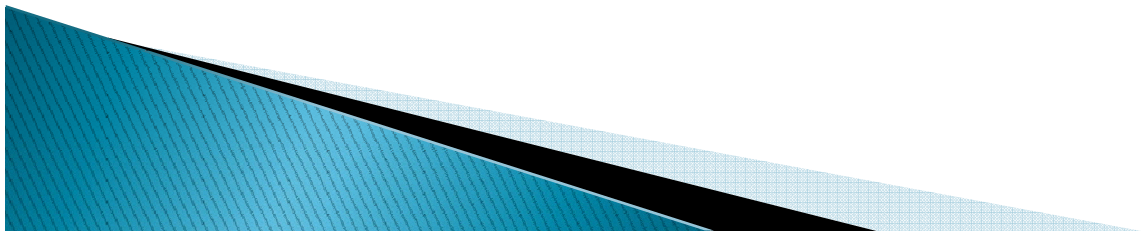
- Antiviral therapy for HIV
- Prevent initial infection of humans cells or spread of the virus after infection
- HIV infects cells by initial binding to CD4 receptor as well as binding of the envelope glycoprotein (gp120) to chemokine coreceptors e.g. CXCR4 of target cell
- siRNA specific to CXCR4 mRNA can downregulate this coreceptor’ s expression on the surface of target cells
- negatively affects HIV fusion

Therapeutic application

- Animal model
- Human blood stem cells injected into mice
- These mice lack their own immune systems, so they tolerate tissue from other species
- Build up a human immune system in mice
- Virus infection
- siRNA can stop T cell destruction by preventing HIV from entering T cell, hence suppressing HIV replication
- Clinical trials in human will begin next year
- replace the harsh drug cocktails currently prescribed to patients with HIV, reducing the side effects of treatment

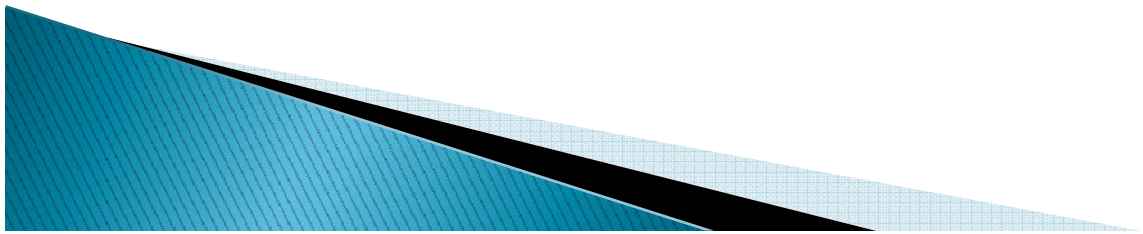
Therapeutic application

- Antiviral therapy for HBV
- Treatment with nucleoside analogs such as lamivudine can only partially inhibit HBV replication
- HBV infection cannot be entirely eliminated due to persistent viral replication
- Synthetic plasmid-based siRNA targeting the surface antigen is able to knock down the HBV expression



Limitations of RNAi-based therapy

- Possibility of off-target effect, silence non-target genes
- Delivery method, inserting foreign vector sequences into chromosomal DNA, insertional activation or inactivation of cellular gene
- siRNA stability, transient effect



Conclusion

- Sequence-specific, diverse applications
- Attractive as a therapeutic agent

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