RNA INTERFERENCE

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Topics to be discussed

- **Motivation**
- **What is RNA Interference?**
- **How RNA Interference Technology works?**
- **What are the challenges to be encountered?**
- **Some existing software based on this technology**

Motivation

- RNAi technology is supposedly next big thing in biotech after recombinant DNA technology
- ***Fortune*** has dubbed RNA interference biotech's next "*billion dollar breakthrough*" in a cover story
- ***Science*** named it the "*breakthrough of the year*" for 2002

Motivation

- **CCMB scientist Utpal Bhadra developed drug for cancer treatment using this technology**

  http://www.thehindubusinessline.com/bline/2004/02/21/stories/2004022102261700.htm

  (media release Feb 20,2004)

- **Ocimum Biosolutions is going to develop software to cater to this technology**

  http://economictimes.indiatimes.com/articleshow/435959.cms (media release January 20 , 2004)

What is RNA Interference?

- It is the mechanism by which double stranded RNA (dsRNA) suppresses the expression of a gene bearing its complementary sequence.
- Successful implementation of this technology requires the design of double stranded RNA (dsRNA) identical to specific regions of the target gene.

Other similar technologies:

- **Please avoid confusion with other similar technologies**

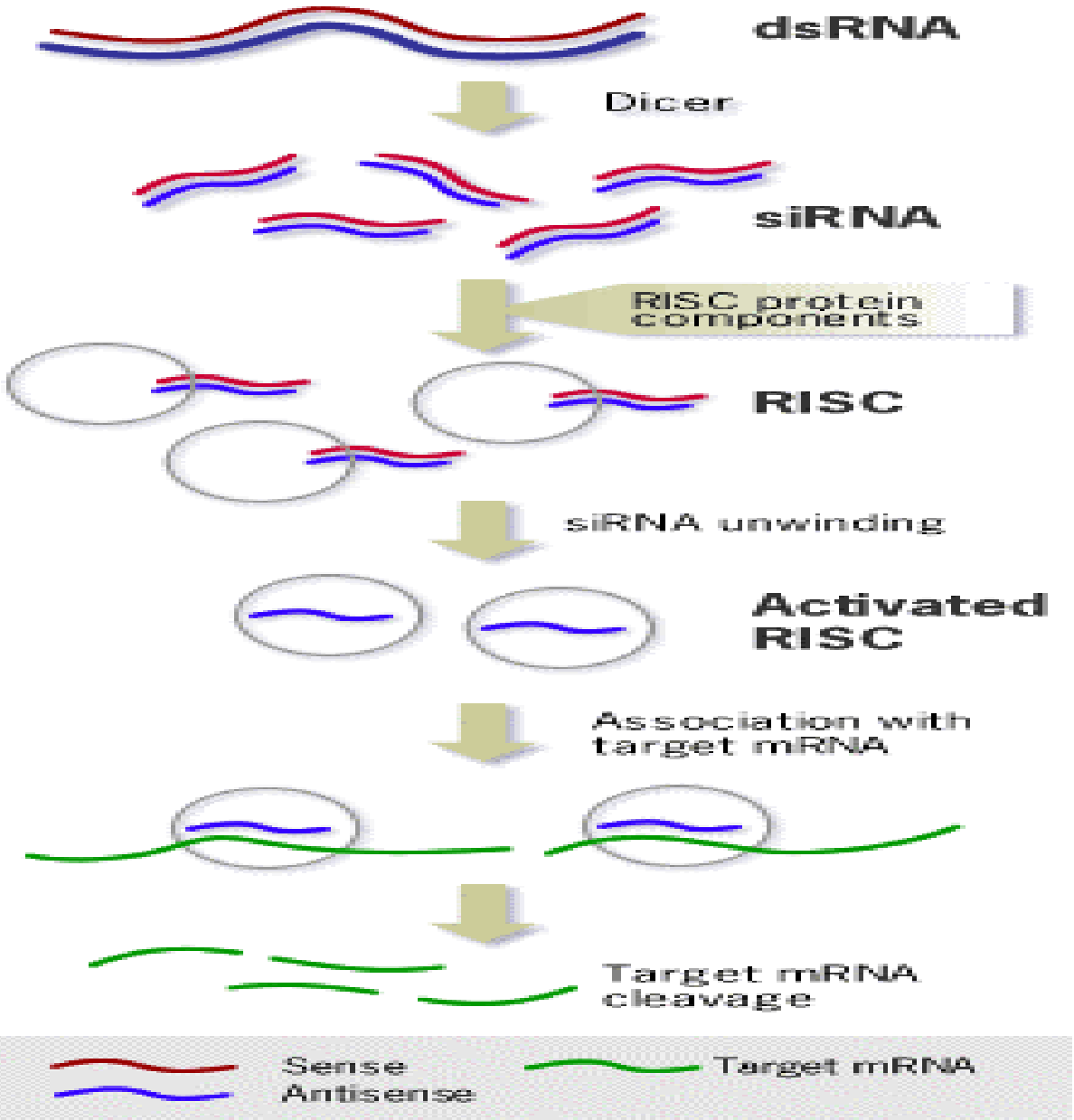
  1. Antisense technology : Interrupts translation of mRNA to protein by introducing single stands of RNA targeted to bind with the mRNA.

  2. Recombinant DNA technology:

  It is the task of cutting DNA from one organism and pasting it into a new organism that reproduces to make proteins of potential therapeutic value

Differences :

Antisense technology	RNAi Interference
1.Single strands of RNA are targeted to bind with the mRNA **2. No RISC complex** **3. Antisense technology is hard to target and unstable in the body** **4. Antisense therapies seek to "block" a key cellular biochemical process**	**1.RNAi mimics a natural process by using double stranded siRNAs.** **2. RISC complex forms** **3.Relatively easy to target** **4. RNAi which "directs" the cell's own biology (RISC complex) to detect and shut off rogue genetic activity and corresponding protein synthesis.**


dsRNA
Dicer
siRNA
RISC protein components
RISC
siRNA unwinding
Activated RISC
Association with target mRNA
Target mRNA cleavage
Sense
Antisense
Target mRNA

How this technology works?

STEP 1:

- Long double-stranded RNAs (>200 nt) with complementary sequence of a part of the target gene is to be introduced
- Upon introduction, the long dsRNAs enter a cellular pathway that is commonly referred to as the RNA interference (RNAi) pathway

How this technology works?

STEP 2:

- The dsRNAs get processed into 20-25 nucleotide (nt) small interfering RNAs (siRNAs) by an RNase III-like enzyme called Dicer (initiation step).

How this technology works?

STEP 3:

The siRNAs assemble into endoribonuclease containing complexes known as RNA-induced silencing complexes (RISCs), unwinding in the process.

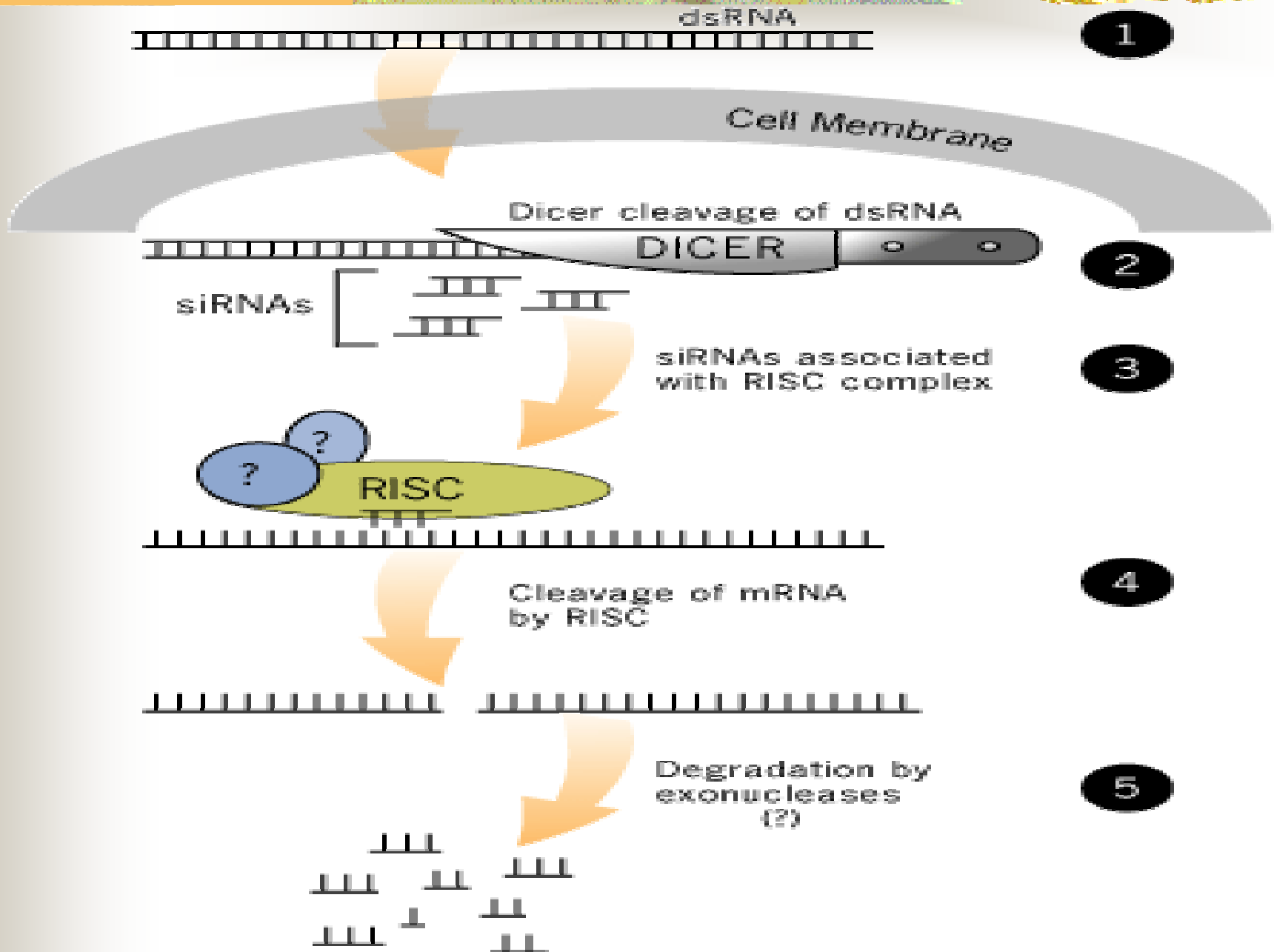
How this technology works?

STEP 4:

- Activated RISC then binds to complementary transcript by base pairing interactions between the siRNA antisense strand and the mRNA.

STEP 5:

- The bound mRNA is cleaved and sequence specific degradation of mRNA results in gene silencing.


dsRNA
1
Cell Membrane
Dicer cleavage of dsRNA
DICER
2
siRNAs
siRNAs associated
with RISC complex
3
?
?
RISC
Cleavage of mRNA
by RISC
4
Degradation by
exonucleases
(?)
5

What problems arise while introducing this method in mammals?

- In mammalian cells, introduction of long dsRNA (>30 nt) initiates a potent antiviral response due to nonspecific inhibition of protein synthesis and RNA degradation.
- Remedy: Introduction of double stranded siRNA instead of dsRNA

What are challenges to be encountered?

- ***1. Validating targets***
- ***2. Delivery of RNAi in mammalian cells.***

Validating targets

Guidelines are proposed by Elbashir

1. **Choose target starting with AA**
2. **Avoid stretches of > 4A's in the target sequence because they act as termination signal for RNA pol III**
3. **Eliminate from consideration any target sequences with more than 16-17 contiguous base pairs of homology to other coding sequences (Use BLAST).**

Some existing software to choose the target sequence on the mRNA:

1. OptiRNAi
2. iRNAi

OptiRNAi

- It is a web-based program to select siRNA sequences.

  http://bioit.dbi.udel.edu/rnai/

OptiRNAi ---- An RNAi Design Tool

Target selection is the critical step for any RNAi experimentation. OptiRNAi predicts optimum RNAi target sequences using the criteria of Elbashir et 2002. The program will generate up to 10 potential siRNA targets with a score indicating how well each matches empirical and theoretical criteria.

Click here for more information.

The preferred length of the siRNA target is 23 (the default value is 23)

Load the sequence file

Browse...

(The sequence can be in either FASTA format or just plain text)

Or paste the sequence here:

(Beginning with the start codon and ending with stop codon is preferred. Coding regions can be obtained by finding the webpage for your gene of interest at NCBI and clicking on the CDS link)

Submit Reset

Here are the target sequences which can be used to design RNAi:

BLAST	No.	Target sequences	Score	GC%	Location
☐	1	AAGGAAGACCTGGCAGAGAAGTC	89	52	442
☐	2	AACAGGAGTGTGAAGGCTCCTTC	89	52	617
☐	3	AATGACCTCTTCCTTCATGCCGA	89	47	646
☐	4	AAGATGTCATGGCTGTGAGGATG	89	47	362
☐	5	AAGGACATCGGAGACTGCTACCT	89	52	277
☐	6	AAGGTTACAGTGTGCACCAGGAA	89	47	683
☐	7	AAGGTGAGGCAGGAGGTGGAGAA	88	56	727
☐	8	AAGGACCAGCCAGGCAGAGACAG	87	60	174
☐	9	AAAGGCCCAGAACCAAGACATGG	86	52	252
☐	10	AAGAGTTTGTGACCCACACTGAC	86	47	470

iRNAi

- Instantaneous recognition of potential target sequences in the DNA of interest
- Highly customizable search and primer settings to support all available systems

Disadvantage:

- Runs only in Mac OS

Links

Links and research papers for RNA interference:

- http://www.ambion.com/techlib/resources/RNAi/
- http://www.ambion.com/techlib/hottopics/rnai/index.html
- http://www.imgenex.com/pdf/Pathway_pg1.pdf
- http://www.imgenex.com/pdf/Pathway_pg2.pdf
- http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=12445391&dopt=Abstract&itool=iconabstr
- http://conferences.computer.org/bioinformatics/CSB2003/SectA.html

Links for the softwares:

OptiRNai software:

http://bioit.dbi.udel.edu/rnai/

Description:

http://portal.acm.org/citation.cfm?id=938042&coll=GUIDE&dl=GUIDE&CFID=17119513&CFTOKEN=91323383

iRNAi software :

http://www.mekentosj.com/irnai/

Thank You