Gene regulation

Lecture No 4: DNA methylation and microRNA

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DNA methylation

DNA methylation is an essential epigenetic mark that controls gene expression.

It is borne by the genetic material but does not influence its sequence.

It can regulate genomic activities and can be maintained through mitosis and meiosis.

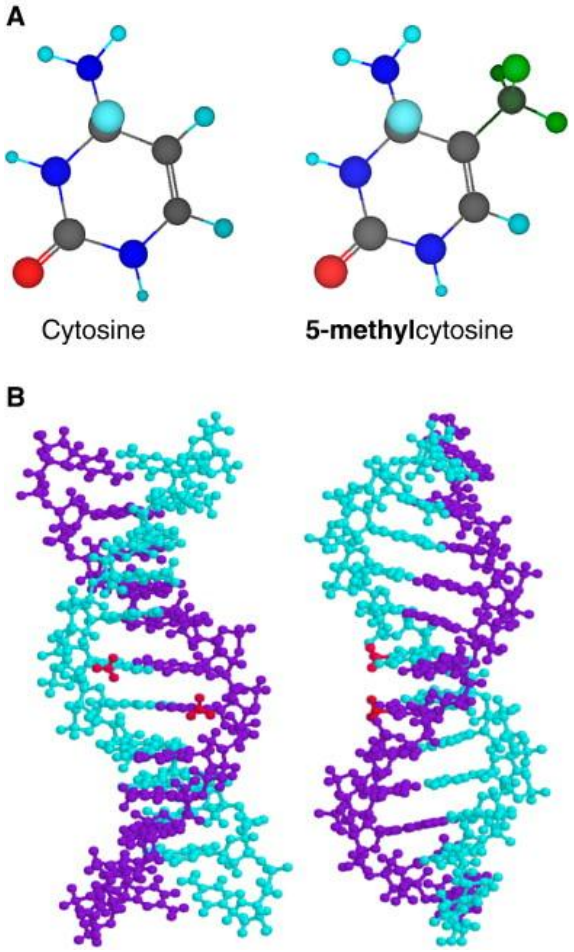
DNA methylation

*Absolutely required for embryonic development.

*The key role of DNA methylation is to control gene expression.

*The DNA of mammals can be methylated on cytosines within the CpG dinucleotides

DNA methylation



DNA Methylation

*The added methyl groups protrude in the major groove of DNA.

* DNA methylation changes the biophysical characteristics of the DNA and inhibits the recognition of DNA by some proteins and permits the binding of others.

DNA Methylation

DNA methyltransferases (DNMTs).

De novo	Maintenance	Cofactor
DNMT3a	DNMT1	DNMT3L
DNMT3b		

DNA Methylation

Proteins that recognize methylated DNA:

Group 1: Methyl-binding Domain (MBD)

Group2: contains the Zinc-finger proteins.
These proteins are bifunctional: they bind methylated DNA, but also some non-methylated consensus sequences.

Group3: bind through their SET-and-RING-Finger-Associated (SRA) domain

All these proteins inhibit gene expression by creating a repressive chromatin structure

DNA Methylation

De novo	Maintenance	Cofactor
MBD	Zinc finger	SRA
MeCP2	Kaiso	UHRF1
MBD1	ZBTB4	UHRF2
MBD2	ZBTB38	
MBD4		

DNA Methylation

The targets of DNAmethylation differ in normal and cancer cells:

First: Parentally imprinted genes.

Second: the transposons.

Third: a number of genes are methylated in a tissue-specific manner.

DNA methylation and Cancer

*DNA methylation is deregulated in cancer

*Abnormal DNA methylation is an early causal event during cellular transformation.

*Demethylating agents can re-establish the expression of silenced tumor suppressor genes and have been approved for clinical use against some leukemias.

Posttranscriptional regulation: microRNA

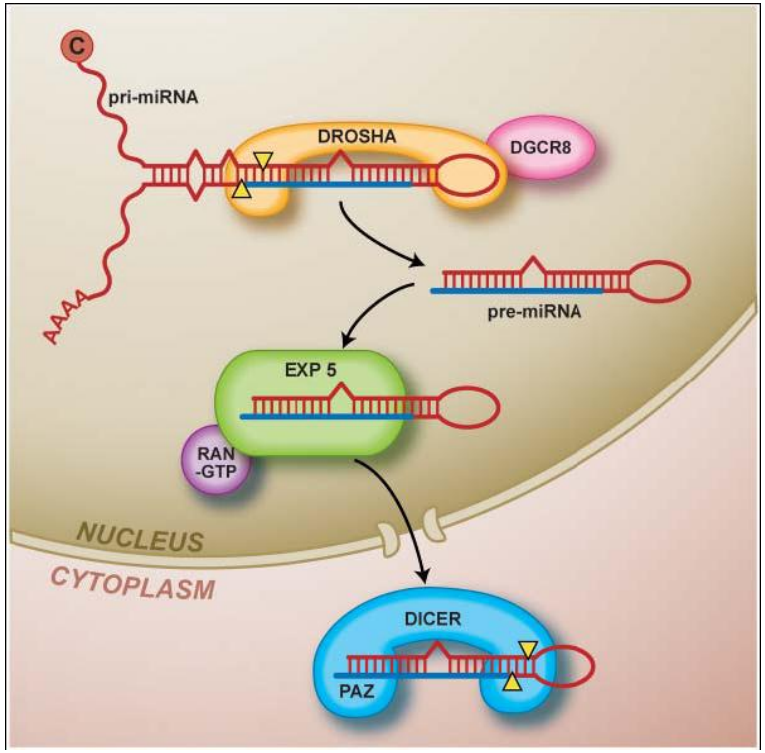
*One of the most important posttranscriptional level of gene regulation.

*It is involved in many molecular interactions, including defense against viruses and regulation of gene expression during development.

*miRNAs are negative regulators that function as specificity determinants, or guides, within complexes that inhibit protein synthesis.

miRNA

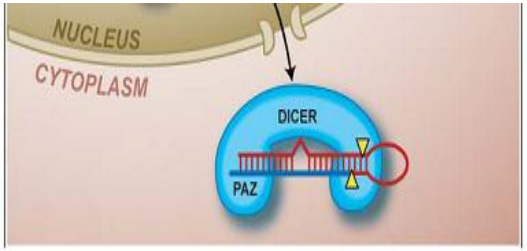
The biogenesis of miRNAs



miRNA

In the Cytoplasm:

pre-miRNAs —DICER→ 21 nt, bearing 2 nt 3` overhang

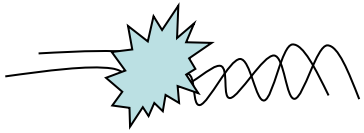


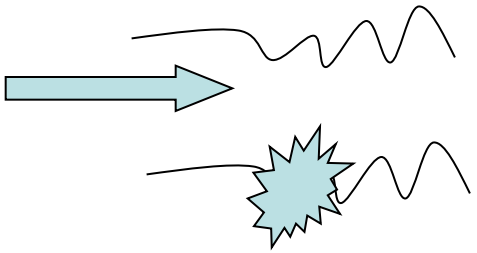
ATP dependent process

phosphorylation of the 5`- ends of the siRNA duplex

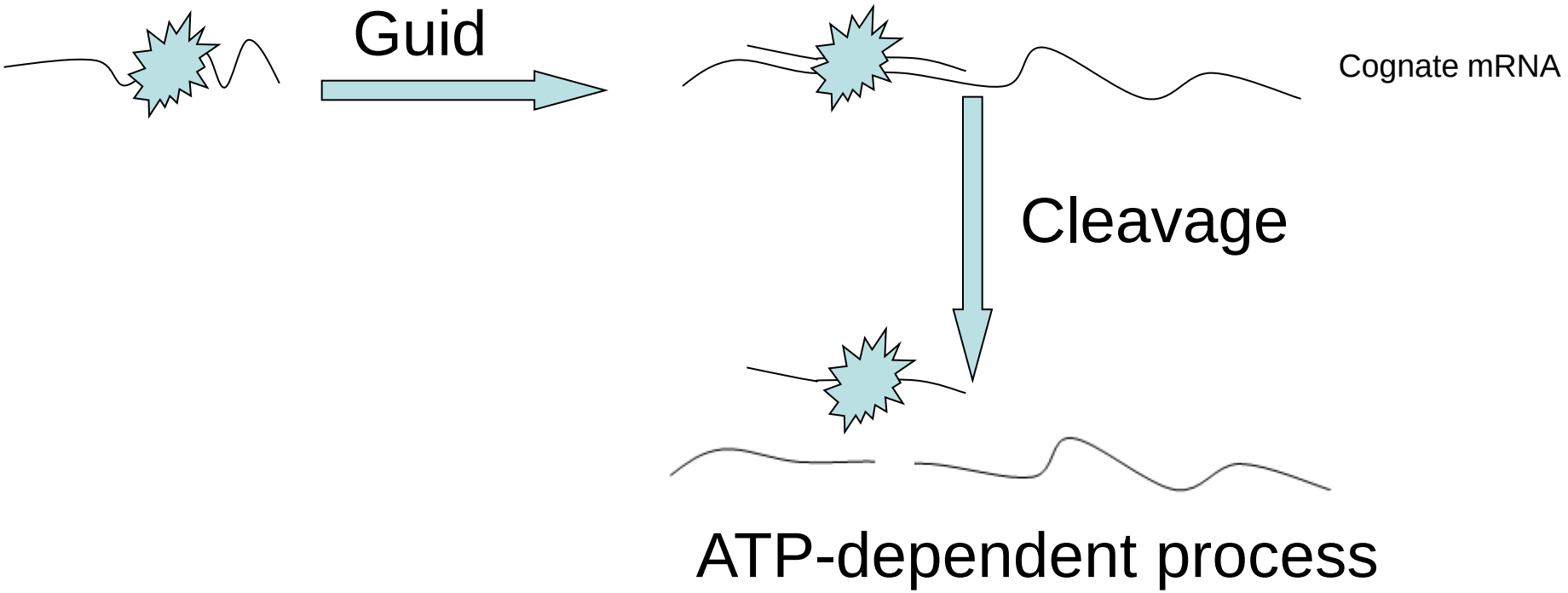
RISC/siRNA complex →

ATP dependent unwinding of the siRNA duplex →





miRNA



RNAi and studying gene function

*RNAi action could be induced in cells

*Introduction of exogenous 21-nucleotide siRNAs or 29mer (29shRNA) duplexes into mammalian cells specifically suppress expression of endogenous and heterologous genes in different mammalian cell lines.

RNAi and studying gene function

siRNA:

*Up to 90% transfection efficiency

29-mer shRNA:

*More potent inducers of RNAi than siRNAs.

*Dicer cleavage of chemically synthesized short hairpin RNAs (shRNAs) with 29-base-pair stem and 2-nucleotide 3` overhangs produces predictable homogeneous small RNAs comprising

the 22 bases at the 3` end of the stem.

RNAi of both siRNA and the 29mer shRNA seems to be sustained for only a limited period of time (up to 72h)

RNAi and studying gene function

shRNA

*By substituting the stem sequences of the mir-30 precursor with unrelated base Paired sequences, could inhibit the expression of mRNA containing a complementary target site.

*These results indicate that the sequence of the precursor does not contribute to miRNA production and thus might be particularly suitable as “vectors” for novel miRNA production.

*These miRNAs can be designed to specifically inactivate the expression of selected target genes in human cells.

*The designed miRNAs can be produced from transfected DNA in human cells and these miRNAs can induce the specific degradation of a cognate mRNA target, similar to transfected siRNAs.

RNAi and studying gene function

Advantages of shRNA:

* Inexpensive and can result in a continous miRNA production, leading to stable inhibition of target mRNA expression

*It is possible to eliminate those cells that have not been transfected with plasmids by selection for antibiotic-resistance genes.

* Viral vectors allow delivery of siRNA expression cassettes into cells at high efficiencies of transfection.

RNAi and studying gene function

Designing siRNA: points to be considered

*G/C content should range between 38% to 52%.

*G/C at the 5` end of the sense strand, and the absence of any GC stretches of more than 9 nt in length induce highly effective gene silencing in mammalian cells.

*Low internal stability of siRNA at the 5` terminus of the antisense strand and at the 3` terminus of the sense strand is prerequisite for effective silencing.

*chemical modifications that stabilize interactions between A–U base pairs, may enhance mRNA targeting efficiency in allele-specific RNAi.

RNAi and studying gene function

* Modifications altering the structure of the A-form major groove of antisense siRNA–mRNA duplexes abolish RNAi.

*siRNA sequences that contain internal repeats or palindromes may form internal fold-back which eventually reduces the silencing potential of the siRNA.

*The efficacy of siRNA is reduced when the target site is embedded within a tight RNA structure.

RNAi and studying gene function

Delivery:

***Calcium phosphate:** (low ph of the buffer, preservation of DNA and DNA-Calcium ratio).

***Liposomes:** cationic lipid reagents have proven to be one of the most efficient methods for the transfection of nucleic acid molecules into cultured cells.

Example: DOTMA (Lipofectine) which is positively charged lipid forms liposomes in aqueous environment for RNA transfection in tissue culture cells.

*The RNA/lipofectin complex can be used to introduce RNA into a wide variety of cells.

*One limitation of the lipofectin procedure is the toxicity associated with the positively charged lipids. For this reason it is prudent to establish the optimal RNA-to-lipofectin ratio for the desired cell type

***Retroviral vectors, reconstituted viral envelopes and electroporation**)

Obstacles of siRNA

*Off target effect

*Low level of expression will resist RNA-mediated interference.

*Interferon response

*hairpin secondary structure in the siRNA expression vectors interferes the standard sequencing reactions.

*The effects of the carriers themselves on cells are not generally known.It should be kept in mind that `antisense' may be caused by the summation of effects of the carrier plus the oligomer, as the carriers dissociate from the oligomer intracellularly.


Questions