Developmental Biology III
(epigenetics)

Anke van Eekelen, PhD
&
Peter Dallas, PhD

Telethon Institute for Child Health Research
Cellular gene expression profiles underlie the development and maintenance of cellular identity.

**Epigenetics:**

The events that change cellular expression profiles without changing the genetic code.

1- chromatin modifications
2- microRNAs
The epi-genotype of each gene is based on histone/DNA modifications:
DNA-packaging in cell nucleus

**Euchromatin**: relaxed packaging structure

**Heterochromatin**: more tightly packed

**Nucleosome** = smallest DNA packaging unit

Histone core: H2A, H2B, H3, H4 proteins

Nucleosome anchor: H1 protein
The epi-genotype of each gene is based on histone/DNA modifications:

- histone acetylation / methylation
- DNA methylation

affect recognition/ access of trans-acting factors to their DNA-binding sites
  (transcription & co-factors)

Alteration of gene expression
Histone (de)acetylation

Acetylation of the N-terminus of aa lysine
→ neutralisation of + charge
→ affinity of DNA for histone ↓↓↓
→ less condensed chromatin → gene transcription ↑↑↑

De-acetylation of lysine N-terminus

Methylation of histone proteins
(aa lysine and/or arginine)

\{ more condensed tightly packed DNA
→ gene transcription ↓↓↓
DNA Methylation

C-5 of cytidine as part of a CpG dinucleotide

Methyl groups (red) in DNA

Access of gene-regulatory proteins to gene promoter regions may be complicated leading to reduced gene expression
DNA Methylation & Cancer

Normal cells: - DNA methylation is concentrated in repetitive regions of the genome
- most CpG islands within gene promoter region are unmethylated!!

Tumor cells: - repetitive DNA loses methylation while CpG islands within gene promoters acquire it!

→ silencing of the associated gene

→ possibly DNMTs are targeted to particular regions via protein-protein interactions within chromatin
Development

Cell differentiation

Transcriptionally active chromatin regions → hyperacetylation & hypomethylation.

But during the process silencing of a region of DNA or a gene may require!

1- chromatin remodeling enzymes (e.g., histone deacetylases) begin gene silencing process.

2- DNA relaxation may recruit DNA methyltransferase resulting in DNA methylation.

3- followed by recruitment of the methyl-CpG binding proteins.

Outcome: The region of DNA will then be heritably maintained in an inactive state.

The epi-genotype is somatically heritable to maintain cellular identity upon differentiation (cellular memory) → Possible because methyl transferase prefers replicated DNA with methyl groups on one of the 2 strands!
II - Micro RNA

mRNA coding for proteins

vs

Small nontranslated RNA:

- tRNAs: adapters in translation
- rRNAs: building ribosomes
- small nuclear RNAs: involved in mRNA splicing
- small nucleolar RNAs: help rRNAs to make ribosomes

- small temporally regulated RNAs = stRNAs
- small interfering RNAs = siRNAs
strRNA

21-25 nt long

mediate downregulation
of gene expression

Target mRNAs after initiation of translation without affecting stability of mRNA

* Known role in control of developmental timing
* Mechanism remains unclear

siRNA

target mRNA and initiate degradation of mRNA

* mechanism explored
* physiological role unclear

microRNA
**stRNA function**: Heterochronic pathway of lin-4 and let-7

* Heterochronic genes are temporal 'equivalents' of homeotic spatial patterning genes

* Prococious mutants: cells inappropriately express later cell fates during early stages of development (lin-41 -/-)

* Retarded mutants: cells reiterate earlier stage fates instead of later wild-type fates (let-7 -/-)
stRNAs
Mechanism of action

- Precursor RNA: Approx 60-70 nt
  - Stem-loop structure
  - processing releases mature stRNA from 5'arm
  - asymmetric cleavage

- mRNA binding sites

- Target mRNAs

Grosshans and Slack, JCB 156, 2002
tRNA is hypothesised to transport precursor RNA to cytoplasm

Pre-miRNA processing

Additional co-factors are believed to recognise the bulged-out nt of mRNA/stRNA complex

Grosshans and Slack, JCB 156, 2002
miRNAs are NOT degraded products of longer RNAs !!!

- identification of miRNAs was based on RNA purification & size selection (verification on Northern blots)

- all miRNAs identified were encoded by genomic regions, for which no genes had been predicted

- surrounding genomic sequence of genes encoding for miRNAs predicted to form a stem-loop structure

- precursor miRNAs accumulate in Dicer -/- animals
Initial discovery of miRNA in C. Elegans, however..

...in 2001: 91 novel miRNAs were identified in 3 organisms
  * C.Elegans
  * Drosophila
  * Humans (to date: hundreds!)
    (*zebrafish)

...some miRNAs seem preserved across phyla
  (perfect homologues)

...also conservation of the polymerase II-like protein Dicer
  (drc-1 in C. Elegans; Dicer in Drosophila; helicoise-MOI in humans)

To date: Posttranscriptional regulatory mechanism of gene expression
  acting in a wide variety of other organisms than C. Elegans
stRNA binding sites in 3' UTR of mRNA

siRNA binding sites anywhere on mRNA

(Banerjee and Slack, BioEssays 24, 2002)
Esquela-Kerscher et al. *Nature Reviews Cancer* 2006; 6, 259–269
RNA interference approach for posttranscriptional gene silencing

Step 1: dsRNA cleavage by Dicer

Step 2: recruitment of siRNA & RNAi factors and formation of RISC

Step 3: siRNA-unwinding and RISC activation

Step 4: mRNA targeting and degradation

RISC: RNA-induced silencing complex

Endogenous /exogenous

Alternative form of genetic manipulation

Tutorial next week!