Third-generation sequencing: a journey to the world of RNA modifications

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Once upon a time... two different cell types

Muscle cell

Nerve cell

How can the same DNA make different cell types?
Once upon a time... two different cell types

Muscle cell

Nerve cell

How can the same DNA make different cell types?

DNA...ATGATCCTCGTAA...ACTAGAGCAT...
Once upon a time... two different cell types

Muscle cell

DNA...
ATGATCTCGTAA...
ACTAGAGCAT...

mRNA...
AUGAUCU...

Polypeptide

A
Once upon a time... two different cell types

![Nerve cell](nerve_cell.png)

**DNA**

```
ATGATCTCGTAA...ACTAGAGCAT...
```

**mRNA**

```
ACUAGAGCA
```

**Polypeptide**

B
But the story gets complicated...

DNA
\[\ldots\text{ATGATCTCGTAA}\ldots\text{ACTAGAGCAT}\ldots\]

mRNA

Polypeptide

\[\text{ACUAGAGCA}\]

B
But the story gets complicated...

**RNA** also has modifications... !!!
RNA modifications may over-rule DNA modifications...!!!
RNA modifications may over-rule DNA modifications...!!!
The same story... in a formal way

DNA → RNA → Protein
The same story... in a formal way

Adapted from Saletore et al., 2012
The same story... in a formal way
The same story... in a formal way

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The same story... in a formal way

Adapted from Saletore et al., 2012

Vogel and Marcotte., 2012
A largely disregarded player: the post-transcriptional regulatory layer
A largely disregarded player: the post-transcriptional regulatory layer

- Translation efficiency
- tRNA availability
- mRNA half life
- Codon usage
- RNA structure

- miRNA activity
- RNA binding proteins
- 5’ RNA degradation
- Ribosome specialization
- RNA modifications (epitranscriptome)

10,000X difference!!
A largely disregarded player: the post-transcriptional regulatory layer

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10,000X difference!!
THE EPITRANSCRIPTOME

PART I: How has our view changed in the last decades?
1950s: RNA modifications fine-tune structure/function

- $N^2,2'-O$-dimethyladenosine (m^2Am)
- $N^4$-methyladenosine (m^A)
- Inosine (I)
- 5-methylcytidine (m^C)
- $N^1$-methyladenosine (m^1A)
- Pseudouridine (Ψ)
- 5-hydroxymethylcytidine (hm^C)
1950-2020: What have we learnt?

1950s: RNA modifications fine-tune structure/function

- \[^{N^0,2'}\text{O-dimethyladenosine (m^2Am)}\]
- \[^{N^0}\text{methyladenosine (m^2A)}\]
- \[^{\text{Inosine (I)}}\]
- \[^{5\text{-methylcytidine (m^2C)}}\]

- \[^{N^0}\text{methyladenosine (m^2A)}\]
- \[^{\text{Pseudouridine (Ψ)}}\]
- \[^{5\text{-hydroxymethylcytidine (hm^2C)}}\]

2011: RNA modifications are reversible!

- \[^{\text{m^6A methyltransferase (writer)}}\]
- \[^{\text{m^6A demethylase (eraser)}}\]

→ Function!

Jia et al., Nat Chem Biol 2011
1950-2020: What have we learnt?

**1950s: RNA modifications fine-tune structure/function**

- $N^{2}$-O-dimethyladenosine ($m^{2}Am$)
- $N^{6}$-methyladenosine ($m^{6}A$)
- Inosine (I)
- 5-methylcytidine ($m^{5}C$)
- $N^{1}$-methyladenosine ($m^{1}A$)
- Pseudouridine (U)
- 5-hydroxymethylcytidine ($hm^{5}C$)

**2011: RNA modifications are reversible!**

- $m^{6}A$ methyltransferase (writer)
- FTO
- $m^{6}A$ demethylase (eraser)

Jia et al., Nat Chem Biol 2011

**2012: First genome-wide method (m6A-Seq)**

Number of publications (PubMed)

- 1975
- 1977
- 1979
- 1981
- 1983
- 1985
- 1987
- 1990
- 1992
- 1994
- 1996
- 1998
- 2000
- 2002
- 2004
- 2006
- 2008
- 2010
- 2012
- 2014
- 2016

- m$^{6}$A
- Ab
1950-2020: What have we learnt?

1950s: RNA modifications fine-tune structure/function

- N²,2'-O-dimethyladenosine (m²Am)
- N⁶-methyladenosine (m⁶A)
- Inosine (I)
- 5-methylcytidine (m⁵C)
- N⁷-methyladenosine (m⁷A)
- Pseudouridine (Up)
- 5-hydroxymethylcytidine (hm⁵C)

2011: RNA modifications are reversible!

Jia et al., Nat Chem Biol 2011

2012: First genome-wide method (m6A-Seq)

2013-2020: Pivotal roles of m6A in cellular functions

- Cell differentiation (2014)
- Stress responses (2015)
- mRNA half lives /RNA stability (2013)
- Sex determination (2016)
- Embryonic development (2017)
1950-2020: What have we learnt?

1950s: RNA modifications fine-tune structure/function

- RNA modifications are reversible!
  - FTO (Jia et al., Nat Chem Biol 2011)

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- Sex determination (2016)
- Embryonic development (2017)

But... what about other modifications?
THE EPITRANSCRIPTOME

PART II: Methods to detect it transcriptome-wide
The epitranscriptome comes in more than 170 flavours

Jonkhout et al, RNA 2017
The epitranscriptome comes in more than 170 flavours

Antibody-based **RIP-Seq**
- Antibody binding
- Immunoprecipitation
- Library preparation

Chemical-based **Chem-Seq**
- Chemical treatment
- Reverse transcription
- Library preparation

Jonkhout *et al.*, RNA 2017
THIRD GENERATION SEQUENCING TECHNOLOGIES

- Direct detection (no RT, no PCR)
- In principle, all RNA modifications
- No custom protocols
- Stoichiometry
- Isoform-specific information
- Multiple RNA modifications at the same type
- PolyA tail lengths
- Information along sequence (e.g. if two modifications are in same transcript)
So… how can we detect RNA modifications from nanopore sequencing data?

RNA modifications cause disruptions in current intensity

Novoa, Mason & Mattick, Nat Rev Mol Cell Biol 2017
So… how can we detect RNA modifications from nanopore sequencing data?

RNA modifications cause disruptions in current intensity

Real data (WT vs KO)

Novoa, Mason & Mattick, Nat Rev Mol Cell Biol 2017

Liu, Begik et al, Nature Comm 2019
Ramirez, Lucas et al., (in prep)
The future is here ... also in your life

MinION
The future is here ... also in your life

MinION

SmigdION
PART III: Why do we care about RNA modifications?
Our lab: Epitranscriptomics and RNA Dynamics

The Epitranscriptome

**WHAT?**
RNA modifications are regulatory *dynamic* features

- FTO ALKBH5
- METTL14 METTL3

Reversible!

Expanding the RNA lexicon

**HOW?**
Characterize and map them using novel approaches and technologies

- **Oxford Nanopore Technologies** *(direct RNA sequencing)*
- **Non-random misincorporation** *(bioinformatics)*
- **Nucleoside Mass Spectrometry**

**WHY?**
To decipher their role in:

- Vertebrate embryogenesis?
- Human disease?
- Intergenerational inheritance?
- RNA structure-function?
RNA modifications in *intergenerational inheritance*
RNA modifications in intergenerational inheritance

Diet-dependent paternally-acquired metabolic disorders
RNA modifications in intergenerational inheritance

Diet-dependent paternally-acquired metabolic disorders

WHERE/HOW IS THIS INFORMATION TRANSMITTED??
RNA modifications in intergenerational inheritance

Diet-dependent paternally-acquired metabolic disorders

Small RNA (30-40nt) in ND and HFD sperm is different!

WHERE/HOW IS THIS INFORMATION TRANSMITTED??

Chen et al, Science 2016; Sharma et al., Science 2016
RNA modifications in sperm RNAs ALSO change with diet

Chen et al., Science 2016; Sharma et al., Science 2016
RNA modifications in intergenerational inheritance

RNA modifications in sperm RNAs ALSO change with diet

RNA modifications are needed to pass the information!

1) Injection of synthetic ‘nude’ tRFs did not cause phenotype, whereas injection of 30-40nt fraction does

2) DNMT2 (-/-) mice cannot pass diet-induced phenotypes across generations!

How does diet convert into sperm RNA modifications?
Role of microbiome?

Aspartic Acid
Glycine
Valine

DNMT2
m5C38

Chen et al, Science 2016; Sharma et al., Science 2016
RNA modifications in viruses
RNA modifications in viruses

• Understand the biology of RNA viruses (dengue, Zika, chikungunya, West Nile)

• Can we *mimic viral RNA strategies* to improve RNAi or ASO properties to improve their stability/evade immune responses within the cell?
RNA modifications in viruses

Viral RNA nanopore sequencing:

- Understand the biology of RNA viruses (dengue, Zika, chikingunya, West Nile)

- Can we **mimic viral RNA strategies** to improve RNAi or ASO properties to improve their stability/evade immune responses within the cell?
RNA modifications in viruses

- Understand the biology of RNA viruses (dengue, Zika, chikingunya, West Nile)
- Can we mimic viral RNA strategies to improve RNAi or ASO properties to improve their stability/evade immune responses within the cell?

Viral RNA nanopore sequencing:

Mass Spectrometry

Collaboration with Juana Diez (UPF)
RNA modifications in cancer
RNA modifications in cancer

Dysregulation of RNA modification enzymes is cancer-specific

**RNAseq (mRNA levels)**

**LAML**

- HENMT1
- AP08E0C3A

**BRCA**

- LAGE3
- TRMT9B

**UCS**

- TRMT112
- HSD17B10
- LAGE3
- HENMT1

**KIRP**

- FBL
- METTL27

Begik et al, *bioRxiv* 2019
Dysregulation of RNA modification enzymes is cancer-specific

RNA modifications in cancer

RNAseq (mRNA levels)

Tumor microarrays (protein levels)

Begik et al, bioRxiv 2019
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Thank you!! 😊

Questions ?