

**N6-methyladenosine-dependent
RNA structural switches
regulate RNA-protein
interactions**

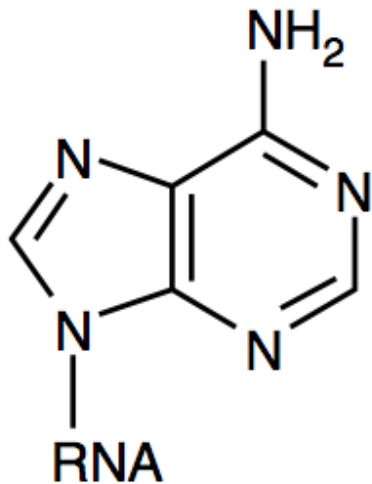
**Marc Parisien
MonBUG
Oct 2016**

Summary of talk:

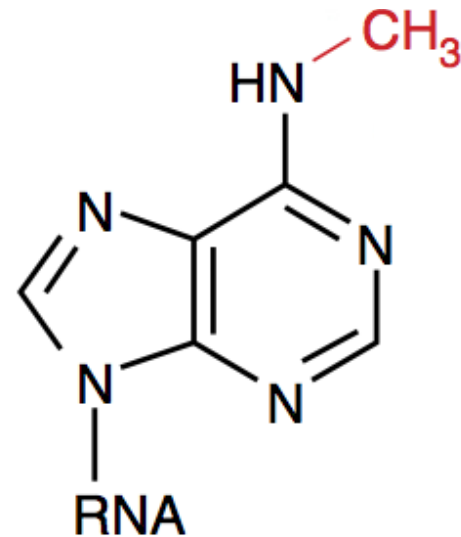
- * What is N6-methyladenosine?
 - * How it affects RNA structure?
-> defining structural switch
 - * Where (genome-wide survey)?
 - * What's in it for the cell?
-

What is N6-methyladenosine?

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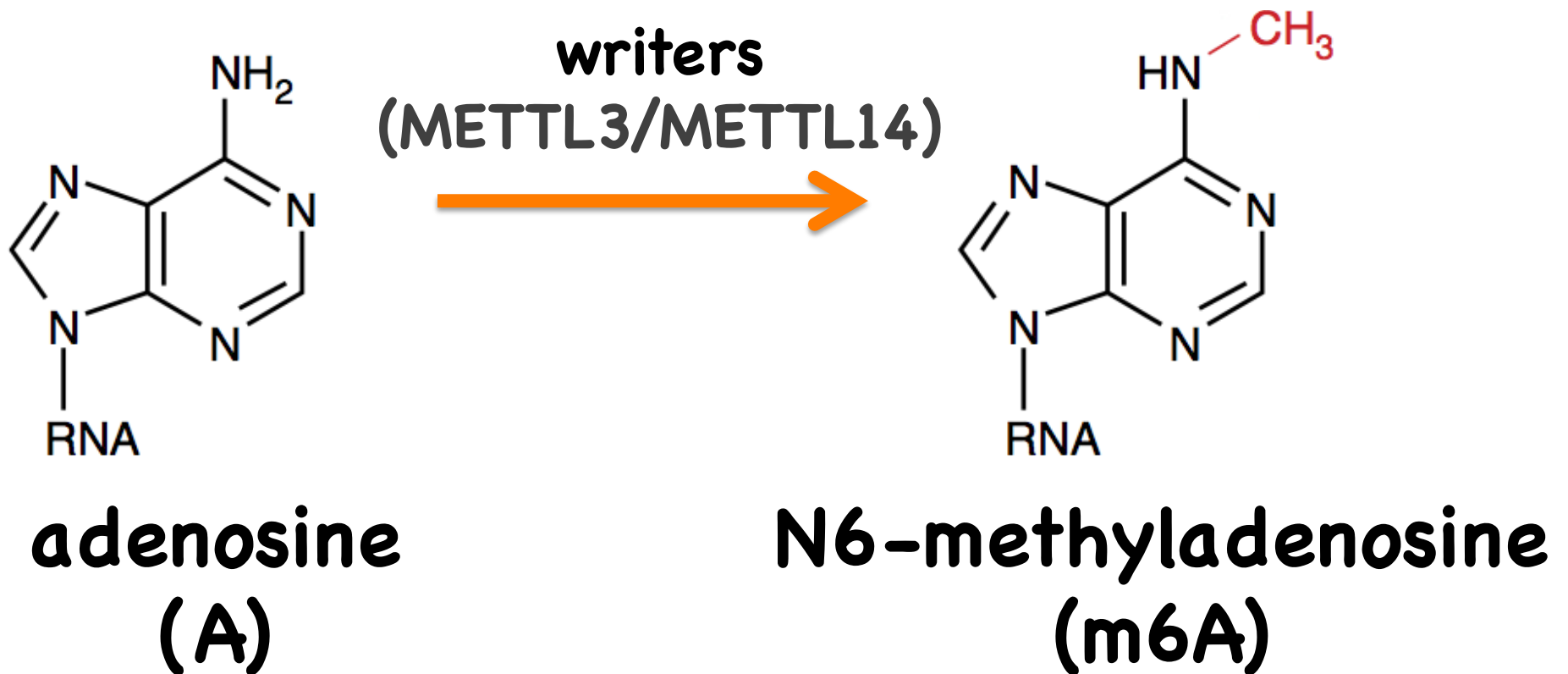


**adenosine
(A)**

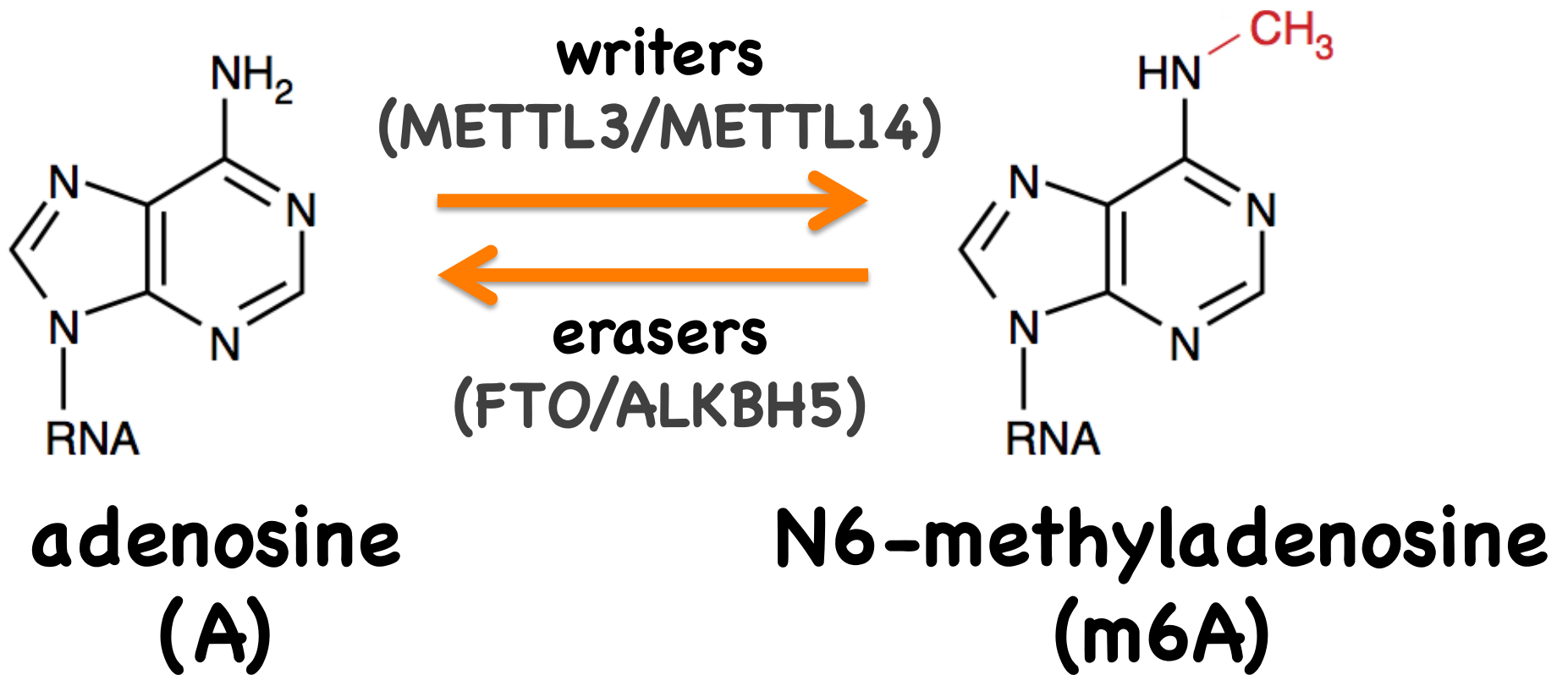


**N6-methyladenosine
(m6A)**

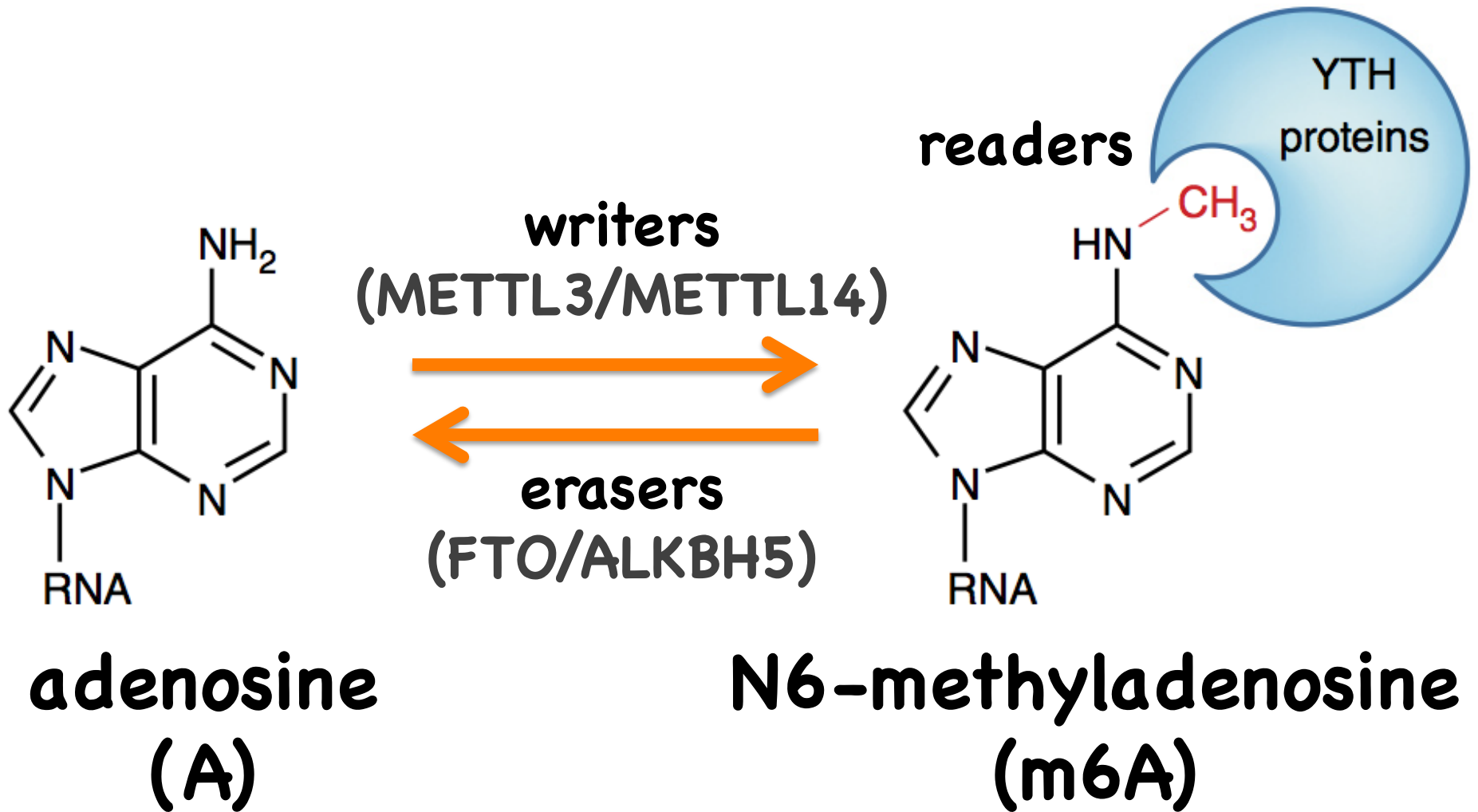
What is N6-methyladenosine?



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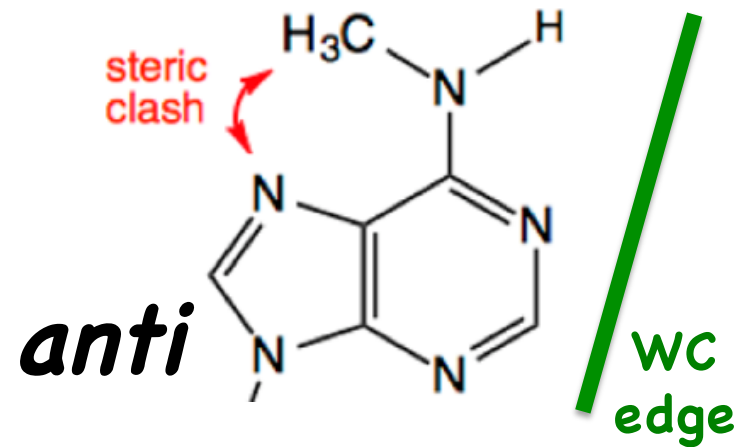
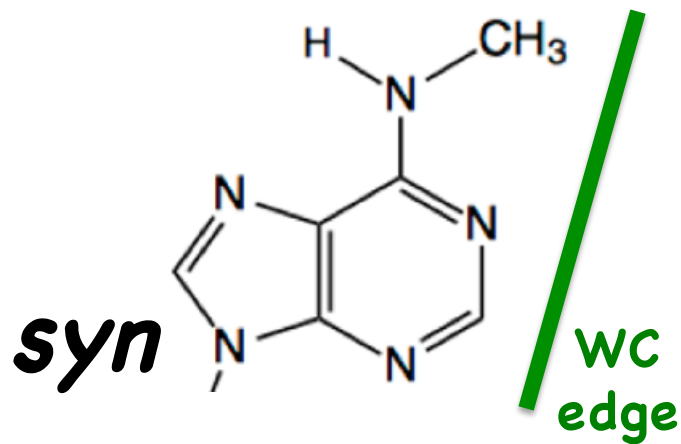


How it affects RNA structure?

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The methyl group (no win):

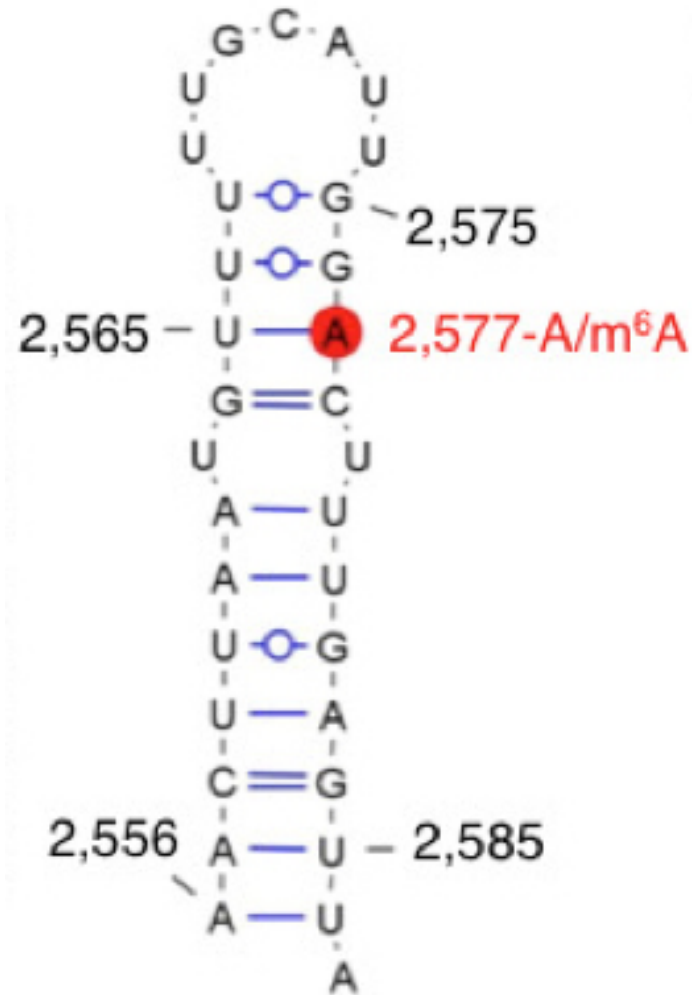
<u>nucleotide</u>	<u>A=U base pair</u>
in <i>syn</i> - allowed	poor H-bond
in <i>anti</i> - steric clash	not ideal geo



How it affects RNA structure?

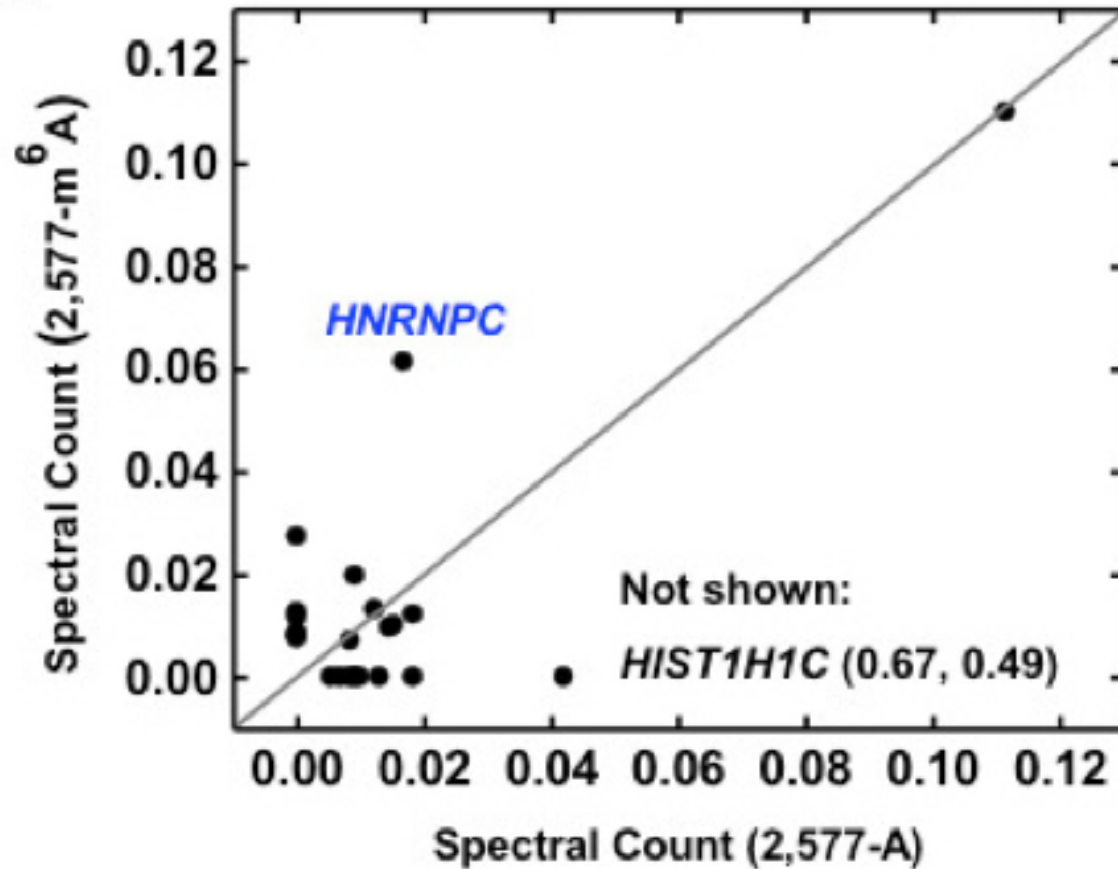
Consider this
RNA hairpin in
the MALAT1
lincRNA:

idea: compare
A vs m⁶A
in whole cell
lysate...



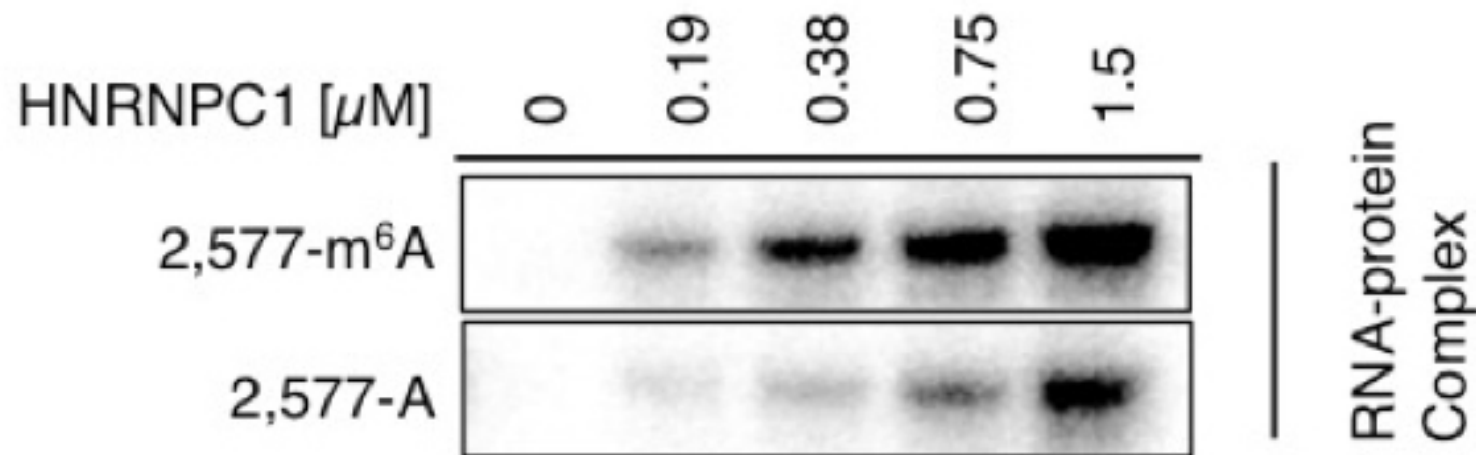
How it affects RNA structure?

Mass Spec.
data of
A vs m⁶A:



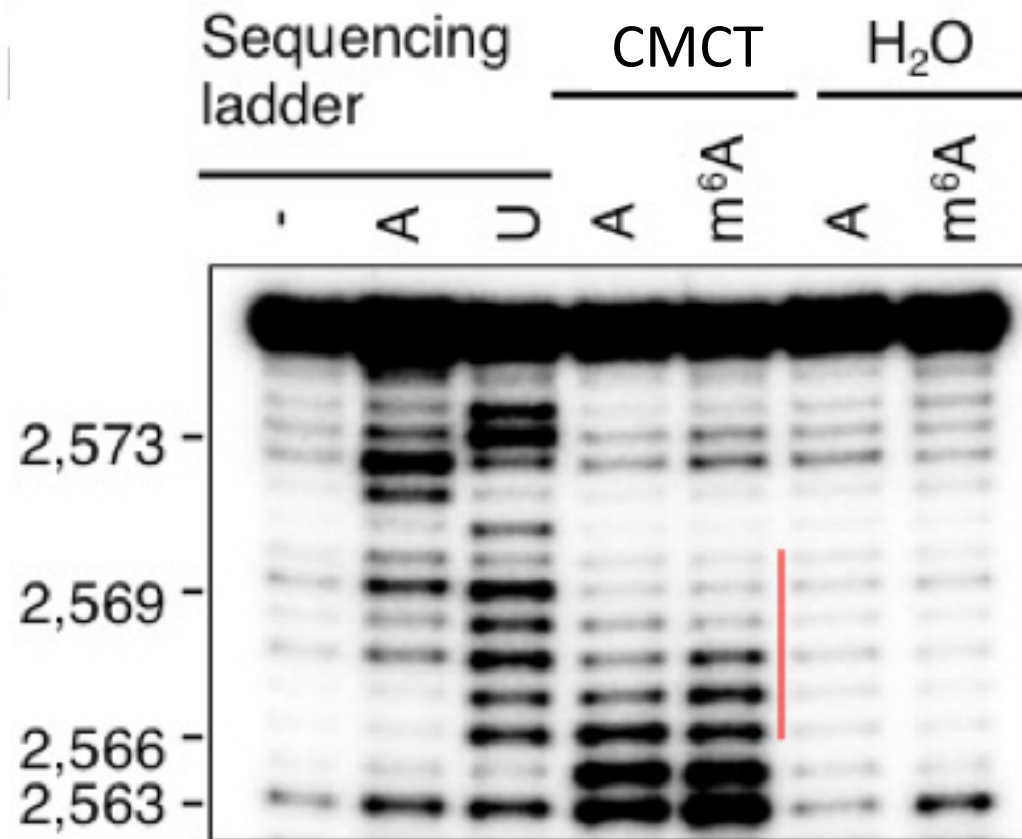
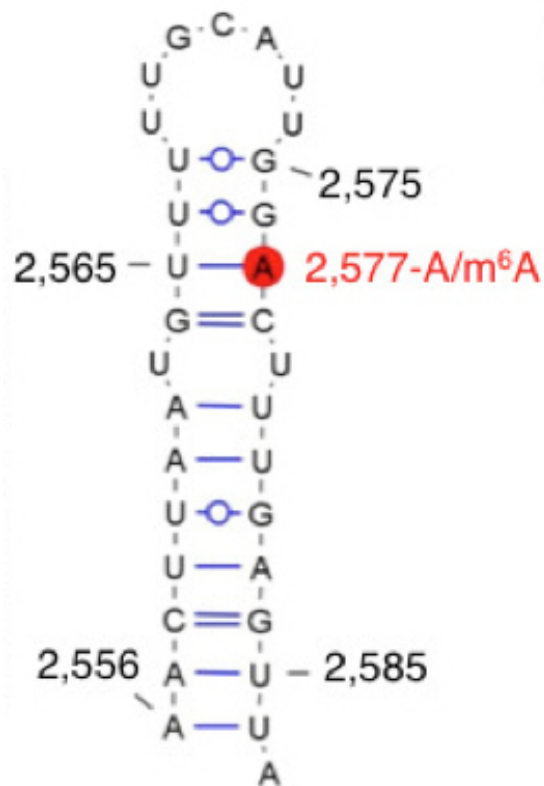
How it affects RNA structure?

the HNRNPC protein binds more to the hairpin upon presence of m⁶A vs A



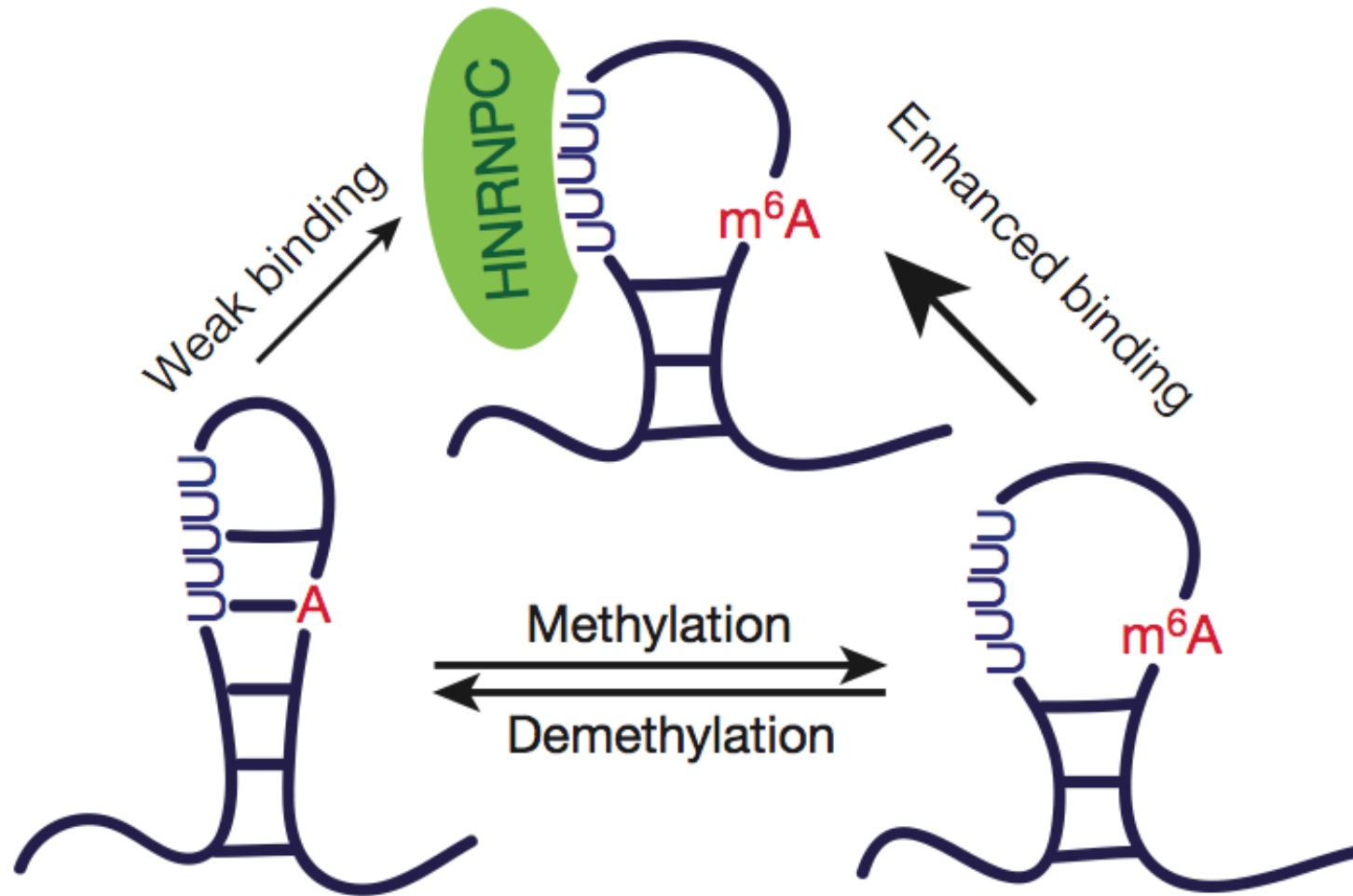
How it affects RNA structure?

Accessibility of the U-tract is enhanced with m6A vs A:



How it affects RNA structure?

an RNA structural switch!



Where (genome-wide survey)?

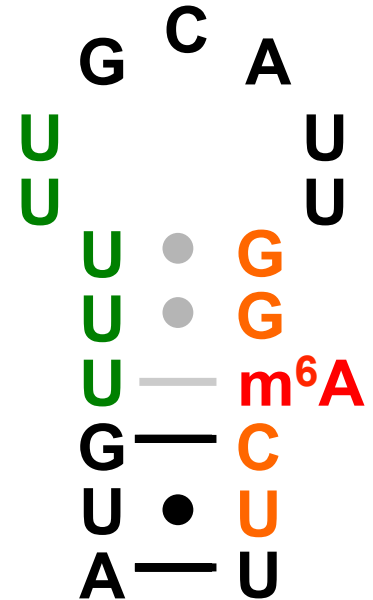
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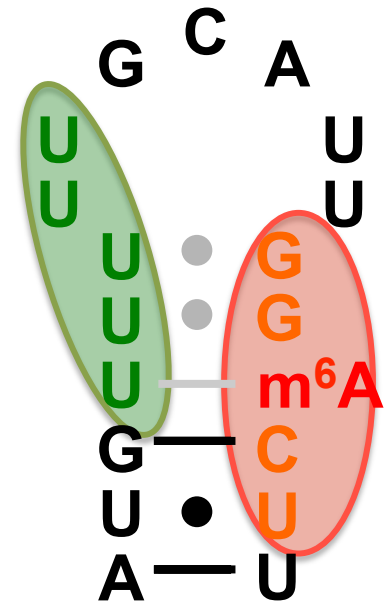
1. Identify potential sites



Where (genome-wide survey)?

Let's find these RNA structural switches, in a genome-wide fashion!

1. Identify potential sites
2. Experimentally confirm:
 - A. Presence of m⁶A
 - B. Presence of HNRNPC



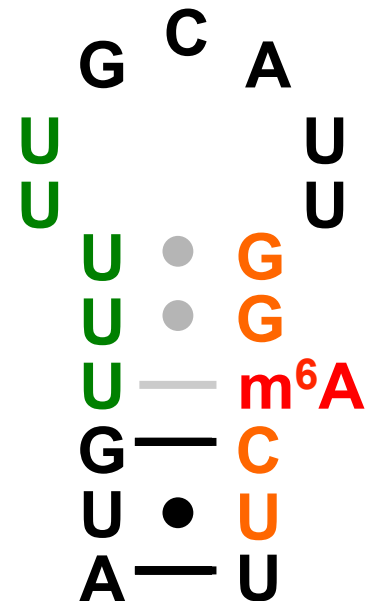
Where (genome-wide survey)?

1. Identify potential sites

HNRNPC binds poly-U tracts
(≥ 5 U's in a row)

m6A has a writer motif used
by the methylases to identify
which sites to methylate:

RRACH (R=A or G, H=not G)

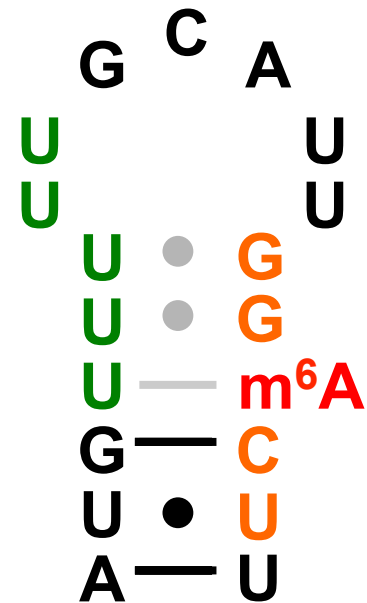


Where (genome-wide survey)?

1. Identify potential sites

search using finite-state automaton
(motifs are linear sequences)

search pre-mRNA sequences
(HNRNPC is a splicing factor)



Where (genome-wide survey)?

1. Identify potential sites

Locate:

```
>MALAT1  
<< TTACCAACTTAATGTTTTTGCATTGGACTTTGAGTTAAGAT >>
```

RRACH: N=78,815,225

```
>MALAT1  
<< TTACCAACTTAATGTTTTTGCATTGGACTTTGAGTTAAGAT >>
```

|<----->|

~ 100 nts

Fold:

```
>MALAT1  
<< TTACCAACTTAATGTTTTTGCATTGGACTTTGAGTTAAGAT >>  
<< ..... ((((((((. (((((((.....)))))))). ))))))) ..... >>
```

Filter:

```
>MALAT1  
<< TTACCAACTTAATGTTTTTGCATTGGACTTTGAGTTAAGAT >>  
<< ..... ((((((((. (((((((.....)))))))). ))))))) ..... >>
```

m⁶A-dependent switch: N=163,946

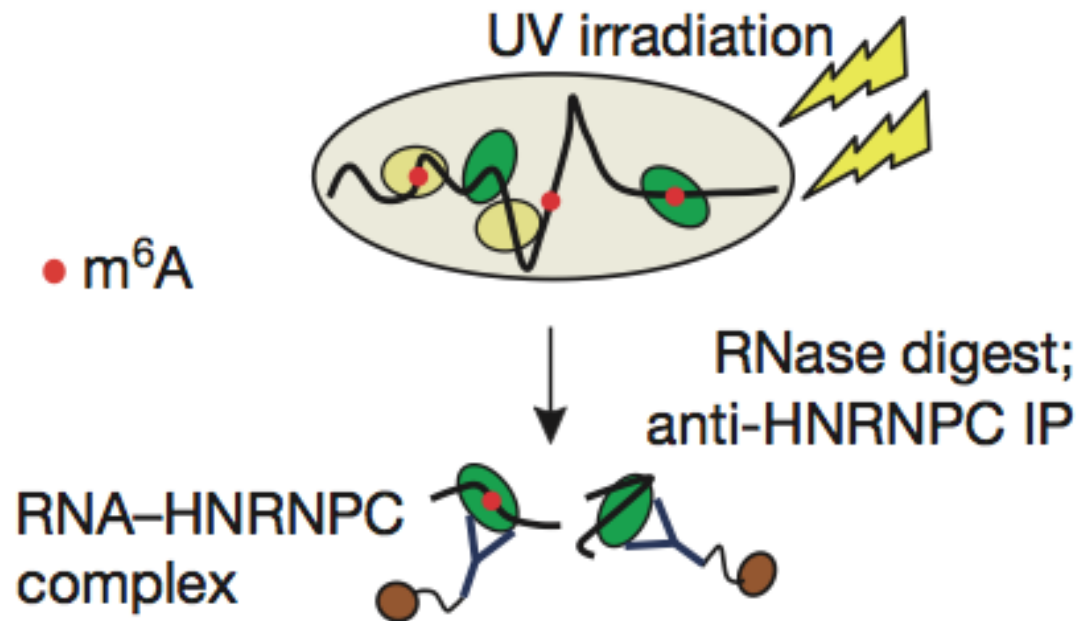
-> A of RRACH paired with one U of poly-U

Where (genome-wide survey)?

**2. Experimentally confirm:
presence of HNRNPC and m6A**

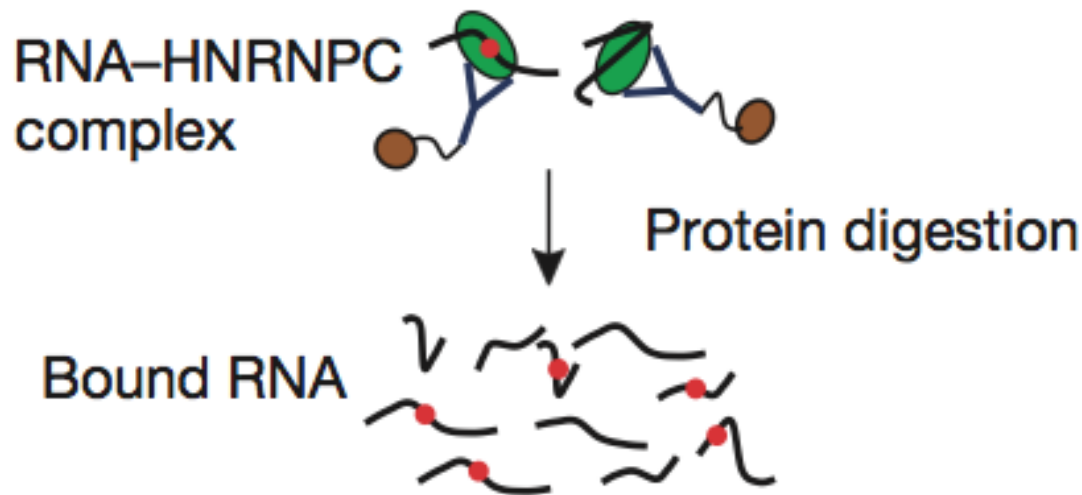
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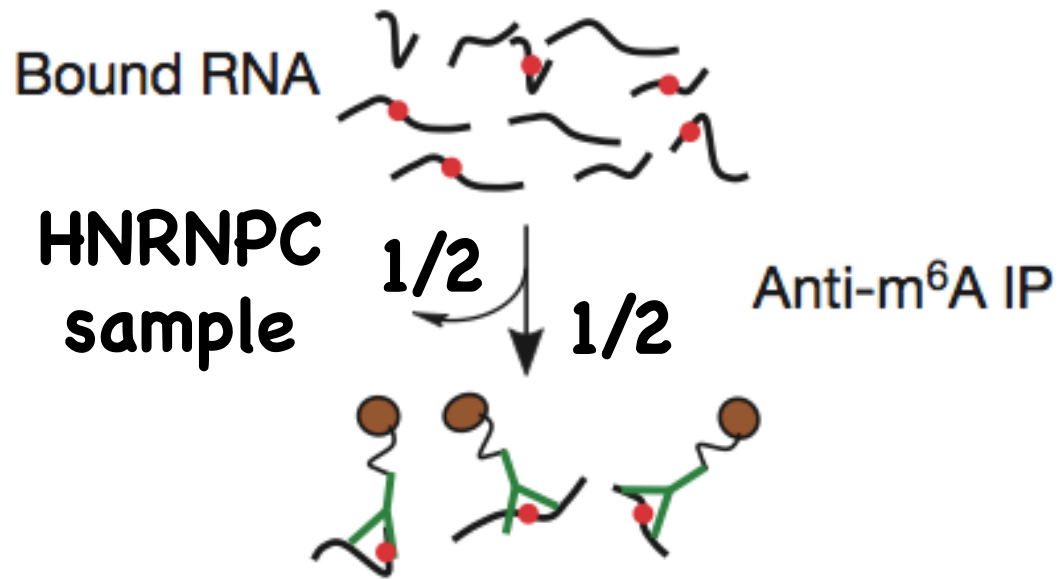
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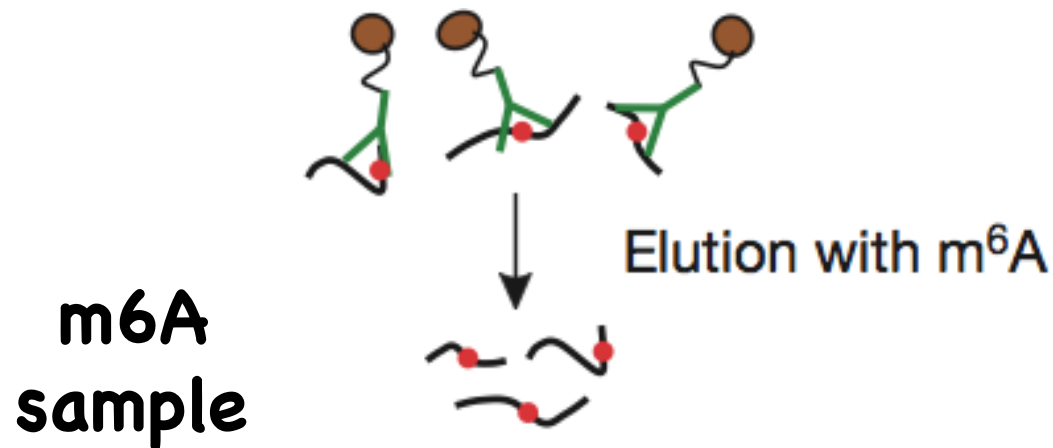
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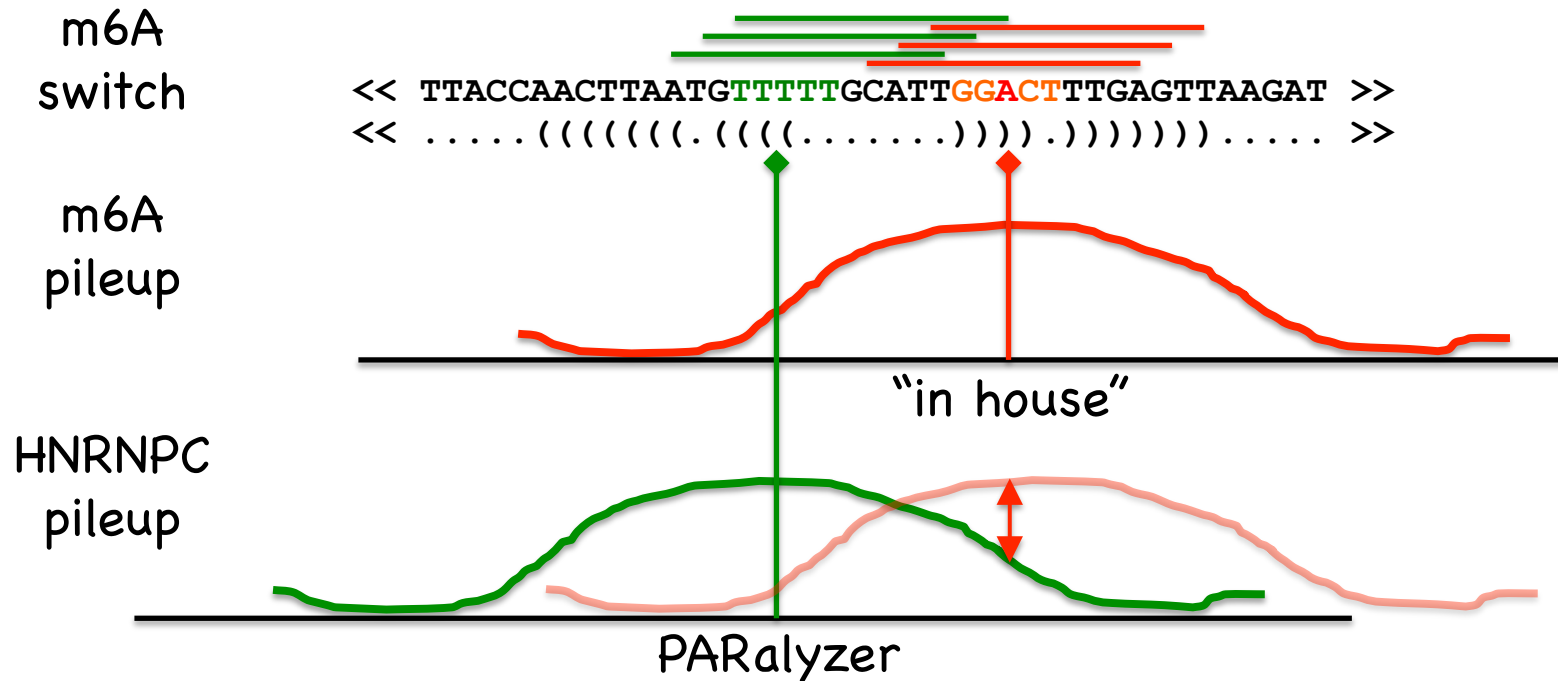
**2. Experimentally confirm:
presence of HNRNPC and m6A**

Both HNRNPC and m6A samples are:

- sent for deep-sequencing**
 - trimmed and mapped on hg19**
 - scanned for "signals"**
-

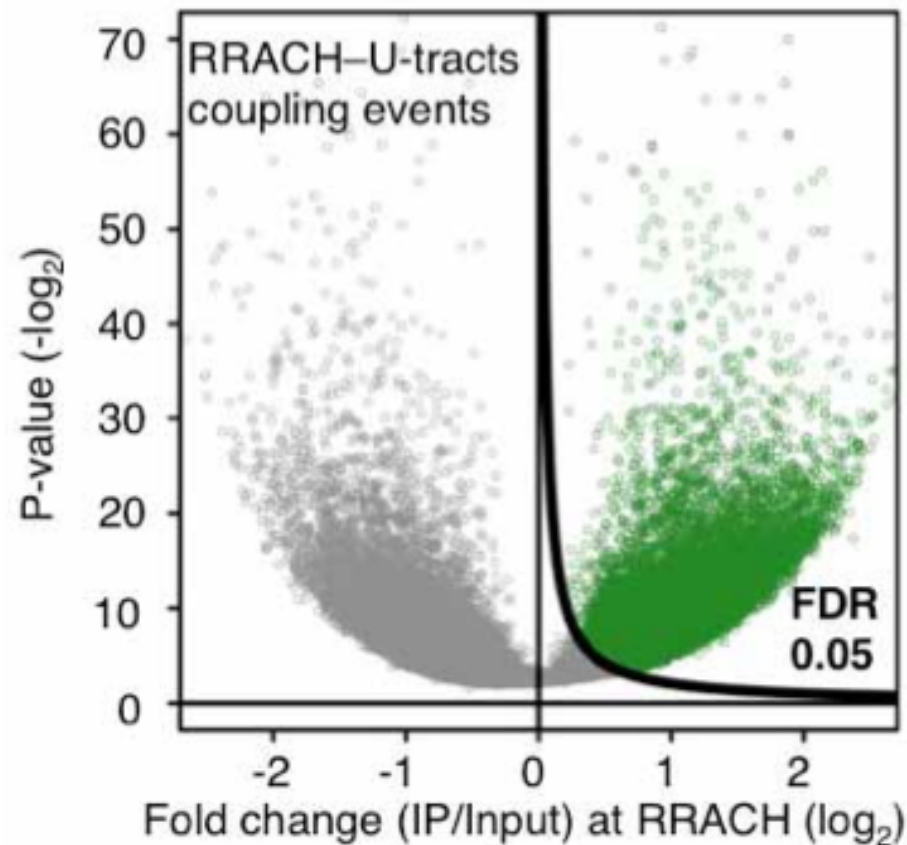
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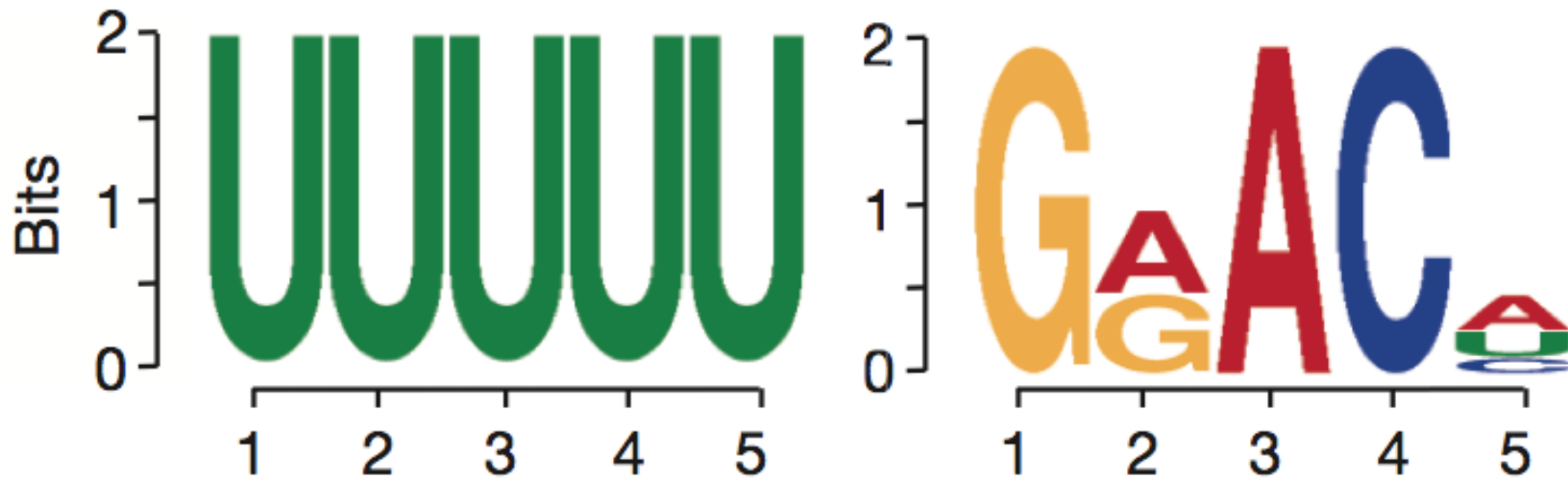
2. Experimentally confirm: presence of HNRNPC and m6A



N=39,060

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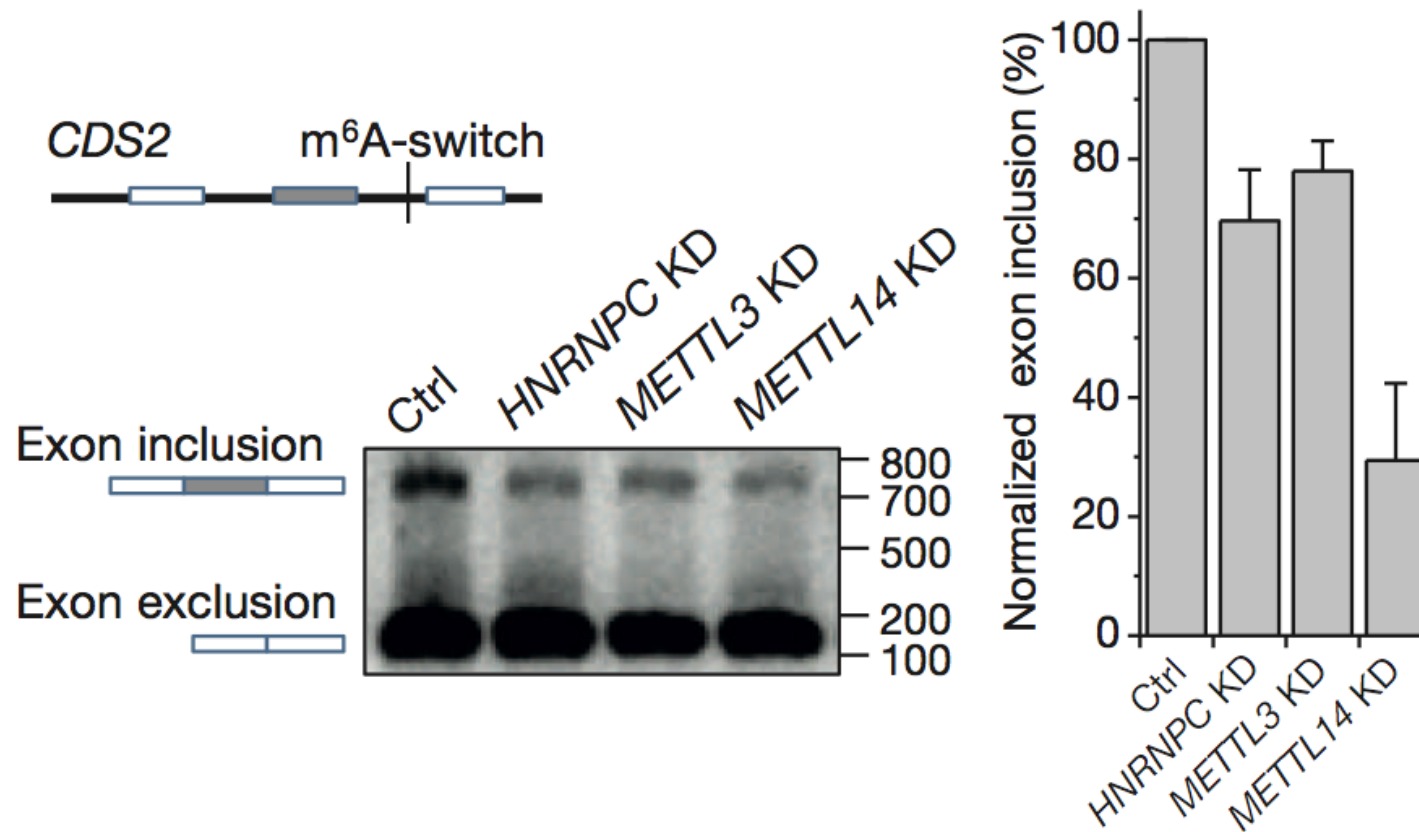
Motif analyses of HNRNPC data (FIRE):



What's in it for the cell?

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Switch sites are located mostly in introns – role in alternative splicing?



To conclude, we looked at:

- * What is N6-methyladenosine**
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- * Where (genome-wide survey)**
- * What's in it for the cell**

Epigenetic regulation at RNA level!

Collaborators:

Nian Liu (Ph.D. project)

Qing Dai (chemistry wizard)

Guanqun Zheng (qRT-PCR)

Chuan He (HHMI)

Tao Pan (RNA Jedi)

Thanks to all!
