人ADARB1与RNA的相互作用关系

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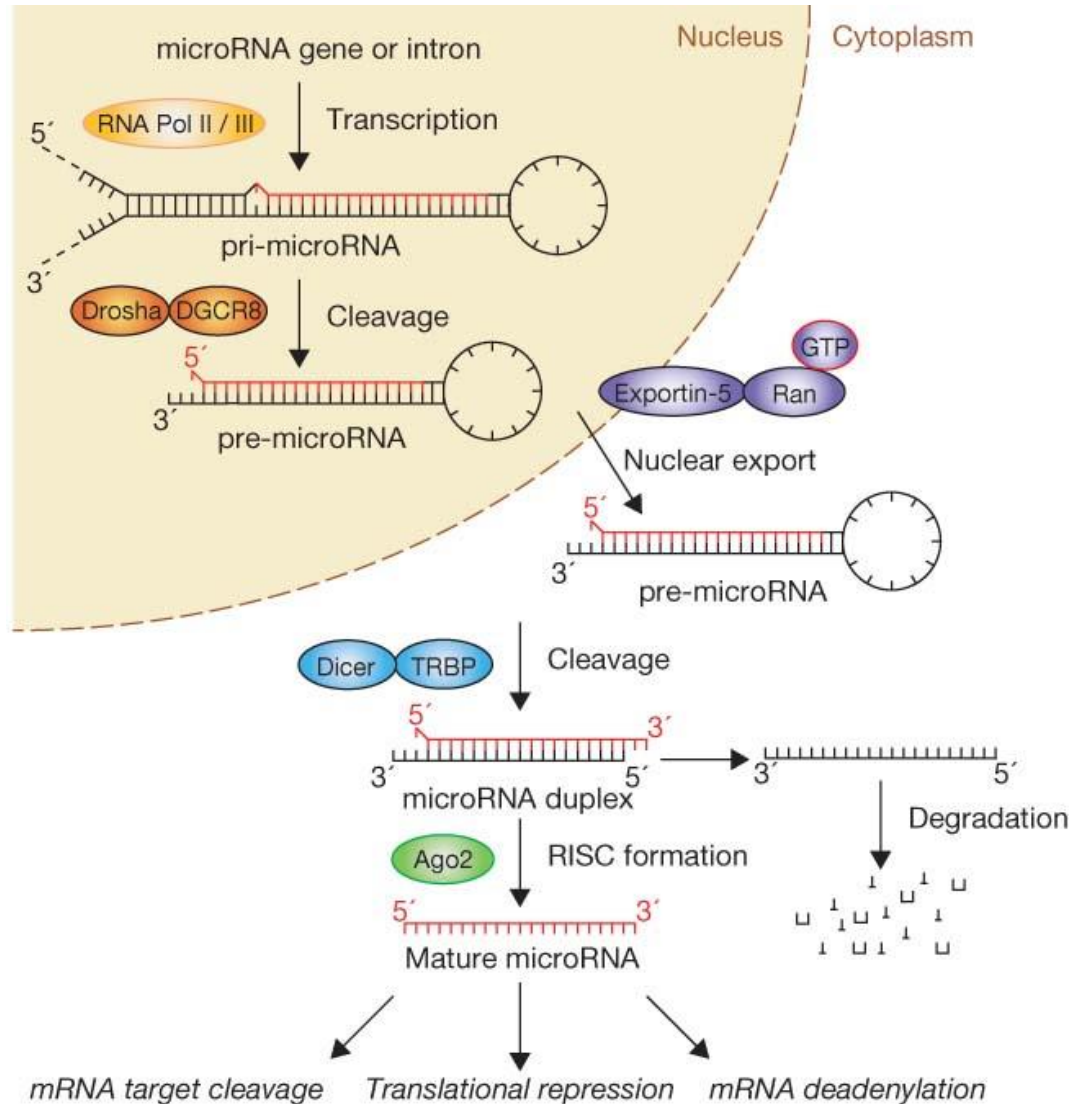导师：汪阳明

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干细胞与非编码核酸实验室
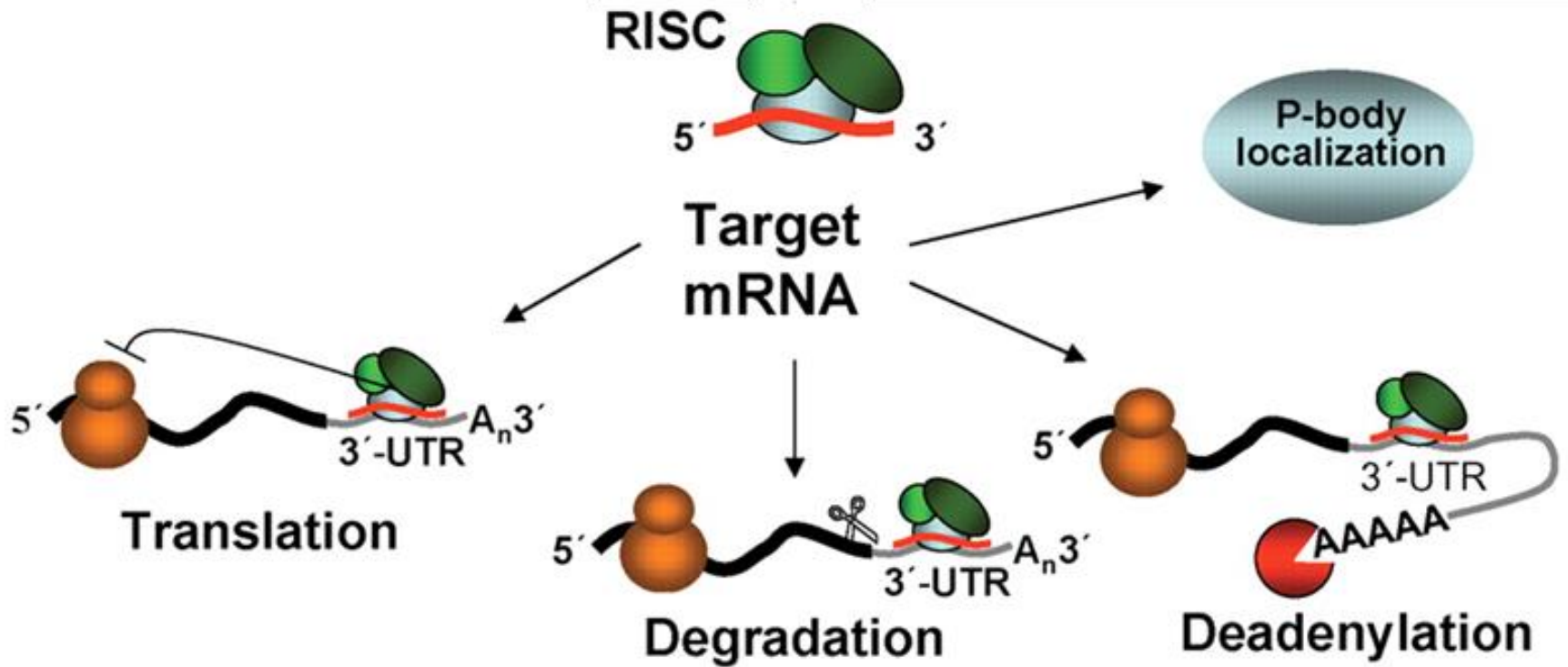
Outline

- 课题背景
  microRNA
  RNA editing

- 课题内容介绍
  研究对象和研究目的
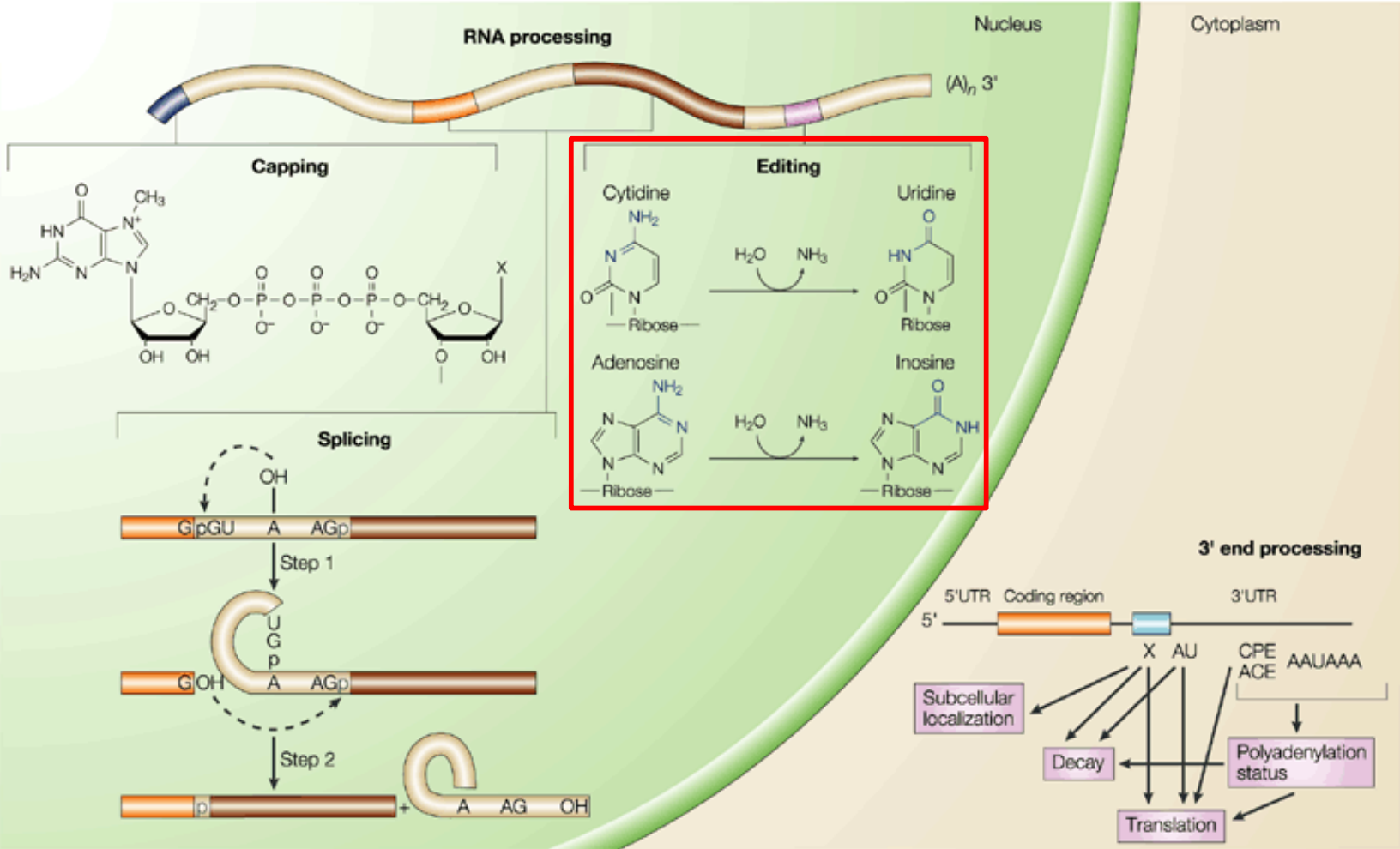 利用生物信息学工具的初步分析

- 小结与讨论

课题背景

microRNA Processing

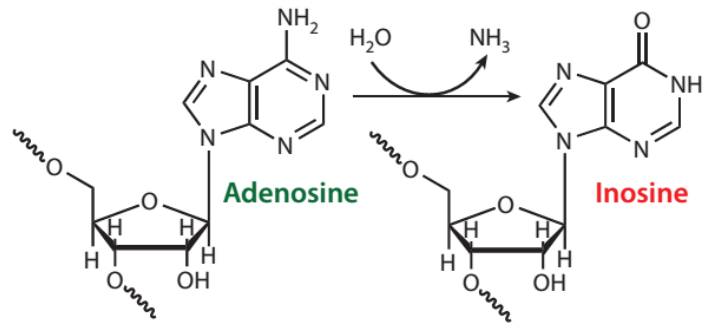# microRNA targeting

RNA editing

研究对象和研究目的

ADARB1 may play a role in microRNA processing and regulation
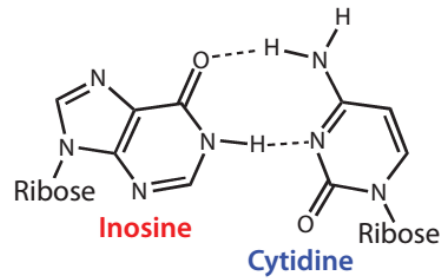
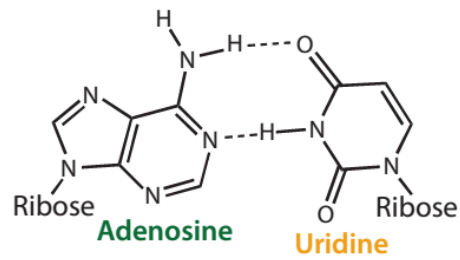## Names and origin

Protein names	Recommended name: **Double-stranded RNA-specific editase 1** EC=3.5.4.37 Alternative name(s): RNA-editing deaminase 1 RNA-editing enzyme 1 dsRNA adenosine deaminase
Gene names	Name: **ADARB1** Synonyms:ADAR2, DRADA2, RED1
Organism	**Homo sapiens (Human)** [Reference proteome]

ADARB1

Uniprot

Browse by taxonomy, keyword, gene ontology, enzyme class or pathway |

Reduce sequence redundancy to 100%, 90% or 50%

Download

Page 1 of 1

Results Customize

› Expand search to "Homo sapiens (Human) [9606]" to include lower taxonomic ranks

› Add columns: Domains

	Entry	Entry name	Status	Protein names	Gene names	Organism	Length
☐	Q9BUB4	ADAT1_HUMAN	⭐	**tRNA-specific adenosine deaminase 1** (hADAT1) (EC 3.5.4.34) (tRNA-specific adenosine-37 deaminase)	**ADAT1**	Homo sapiens (Human)	502
☐	P55265	DSRAD_HUMAN	⭐	**Double-stranded RNA-specific adenosine deaminase** (DRADA) (EC 3.5.4.37) (136 kDa double-stranded RNA-binding protein) (p136) (Interferon-inducible protein 4) (IFI-4) (K88DSRBP)	**ADAR** ADAR1 DSRAD G1P1 IFI4	Homo sapiens (Human)	1,226
☐	P78563	RED1_HUMAN	⭐	**Double-stranded RNA-specific editase 1** (EC 3.5.4.37) (RNA-editing deaminase 1) (RNA-editing enzyme 1) (dsRNA adenosine deaminase)	**ADARB1** ADAR2 DRADA2 RED1	Homo sapiens (Human)	741
☐	Q9NS39	RED2_HUMAN	⭐	**Double-stranded RNA-specific editase B2** (EC 3.5.-.-) (RNA-dependent adenosine deaminase 3) (RNA-editing deaminase 2) (RNA-editing enzyme 2) (dsRNA adenosine deaminase B2)	**ADARB2** ADAR3 RED2	Homo sapiens (Human)	739
☐	Q96M93	ADAD1_HUMAN	⭐	**Adenosine deaminase domain-containing protein 1** (Testis nuclear RNA-binding protein)	**ADAD1** TENR	Homo sapiens (Human)	576
☐	Q8NCV1	ADAD2_HUMAN	⭐	**Adenosine deaminase domain-containing protein 2** (Testis nuclear RNA-binding protein-like)	**ADAD2** TENRL	Homo sapiens (Human)	583

ADAR蛋白的功能：

1.很多研究表明ADAR蛋白之间会形成同源或异源二聚体，共同发挥作用。

2. 改变氨基酸序列，影响蛋白功能（多发生在中枢神经系统）

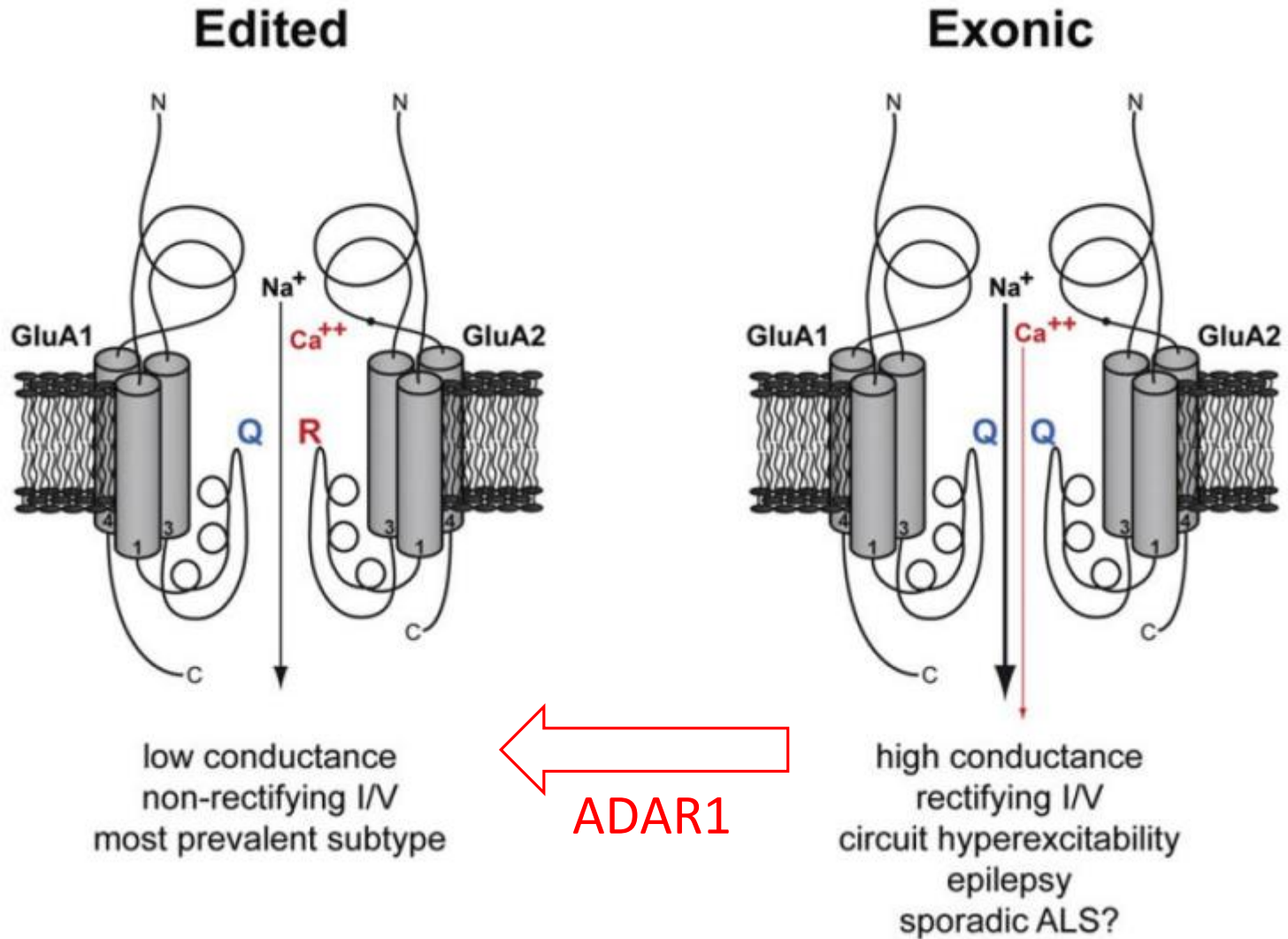GluR, 5-HT2C, Kv1.1, GABAA

3.改变非编码核酸序列，影响转录后切割。
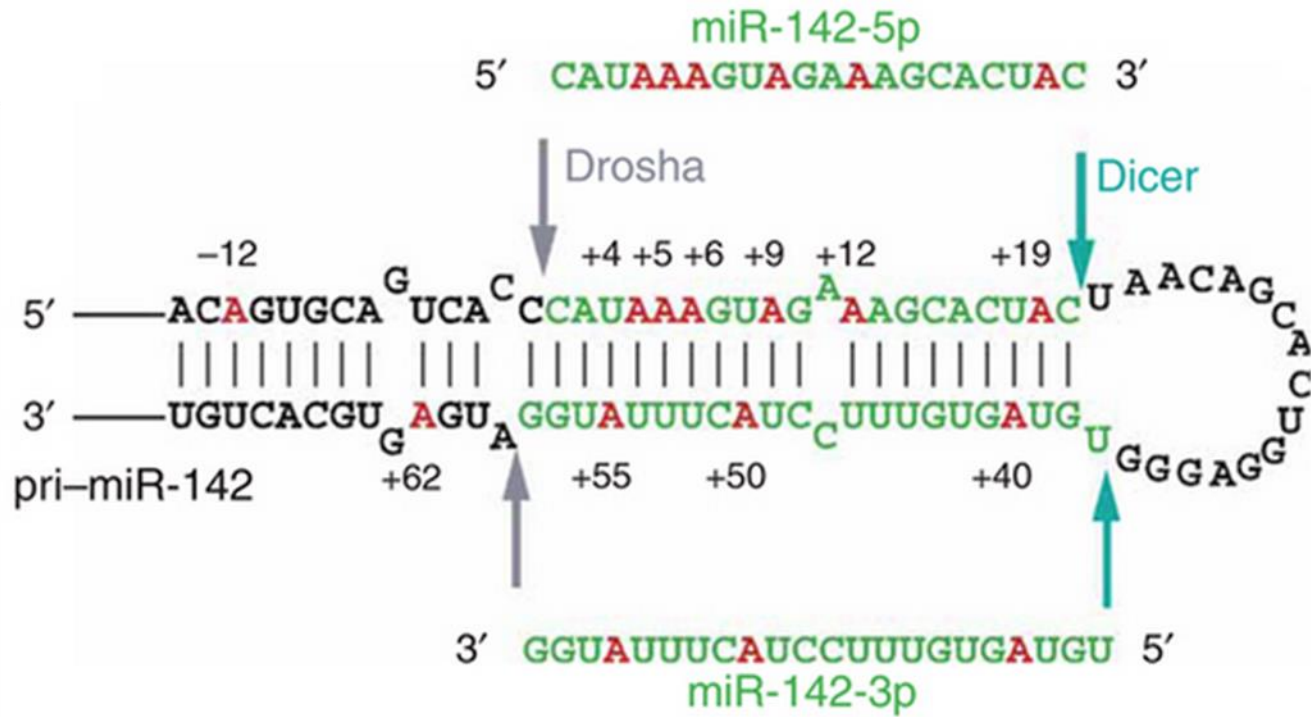
Pri-miR-142, pre-miR-151

4.修饰病毒RNA,影响病毒复制。 ADAR1会被Interferon-β诱导，又被称为Interferon-inducible protein 4 。

丙肝病毒(HCV)，艾滋病病毒（HIV）

5.ADAR3由于缺少editing活性，与其他ADAR蛋白竞争RNA，反而起到了dominant negative的作用。

ADAR1 edits the coding sequence of mRNA

Edited

low conductance
non-rectifying I/V
most prevalent subtype

ADAR1

Exonic

high conductance
rectifying I/V
circuit hyperexcitability
epilepsy
sporadic ALS?

Cell. 1993 Dec 31;75(7):1361-70.

Nat Struct Mol Biol. 2006 January; 13(1): 13–21.

研究目的与猜想：

1.RNA editing可能是microRNA processing中的一种重要的调控方式。

2.通过生物信息学的分析，深入了解ADARB1的序列、结构特征，希望可以利用生物信息学的方法，对ADARB1调控的RNA做一些预测。

protein sequence alignment

Mouse VS Human ADAR1 protein

Pairwise Alignment Result

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
711	3466.5	665/711 (93.5%)	680/711 (95.6%)	10/711 (1.4%)

Mouse VS Rat ADAR1 protein

Pairwise Alignment Result

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
711	3670.0	704/711 (99.0%)	707/711 (99.4%)	0/711 (0.0%)

Human VS Rat ADAR1protein

Pairwise Alignment Result

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
711	3465.5	666/711 (93.7%)	680/711 (95.6%)	10/711 (1.4%)

Mega 6.0
phylogeny

ADAR1

Sequence annotation (Features)

	Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
Molecule processing						
☐	Chain	1 – 1226	1226	Double-stranded RNA-specific adenosine deaminase		PRO_0000171774
Regions						
☐	Repeat	133 – 202	70	DRADA 1		
☐	Repeat	293 – 360	68	DRADA 2		
☐	Domain	503 – 571	69	DRBM 1		
☐	Domain	614 – 682	69	DRBM 2		
☐	Domain	726 – 794	69	DRBM 3		
☐	Domain	886 – 1221	336	A to I editase		
☐	DNA binding	169 – 195	27			
Sites						
☐	Active site	912	1	Proton donor By similarity		
☐	Metal binding	910	1	Zinc By similarity		
☐	Metal binding	966	1	Zinc By similarity		
☐	Metal binding	1036	1	Zinc By similarity		

ADAR2

Sequence annotation (Features)

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
Molecule processing					
☐ Chain	1 – 741	741	Double-stranded RNA-specific editase 1		PRO_0000171779
Regions					
☐ Domain	78 – 144	67	DRBM 1		
☐ Domain	231 – 298	68	DRBM 2		
☐ Domain	370 – 737	368	A to I editase		
☐ Region	83 – 88	6	Interaction with substrate RNA By similarity		
☐ Region	104 – 105	2	Interaction with substrate RNA By similarity		
☐ Region	237 – 242	6	Interaction with substrate RNA By similarity		
☐ Region	259	1	Interaction with substrate RNA By similarity		
Sites					
☐ Active site	396	1	Proton donor		
☐ Metal binding	394	1	Zinc		
☐ Metal binding	451	1	Zinc		
☐ Metal binding	556	1	Zinc		

ADAR3

Sequence annotation (Features)

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
Molecule processing					
☐ Chain	1 – 739	739	Double-stranded RNA-specific editase B2	████████████	PRO_0000171782
Regions					
☐ Domain	125 – 191	67	DRBM 1	─█───	
☐ Domain	274 – 341	68	DRBM 2	──█──	
☐ Domain	408 – 735	328	A to I editase	───██─	
Sites					
☐ Active site	434	1	Proton donor By similarity	───│──	
☐ Metal binding	432	1	Zinc By similarity	───│──	
☐ Metal binding	490	1	Zinc By similarity	───│──	
☐ Metal binding	555	1	Zinc By similarity	────│─	

DRBM (double-strand RNA binding motif)

DRBM of ADAR

DRBM of ADARB1 and other RNA binding proteins

protein-protein interaction
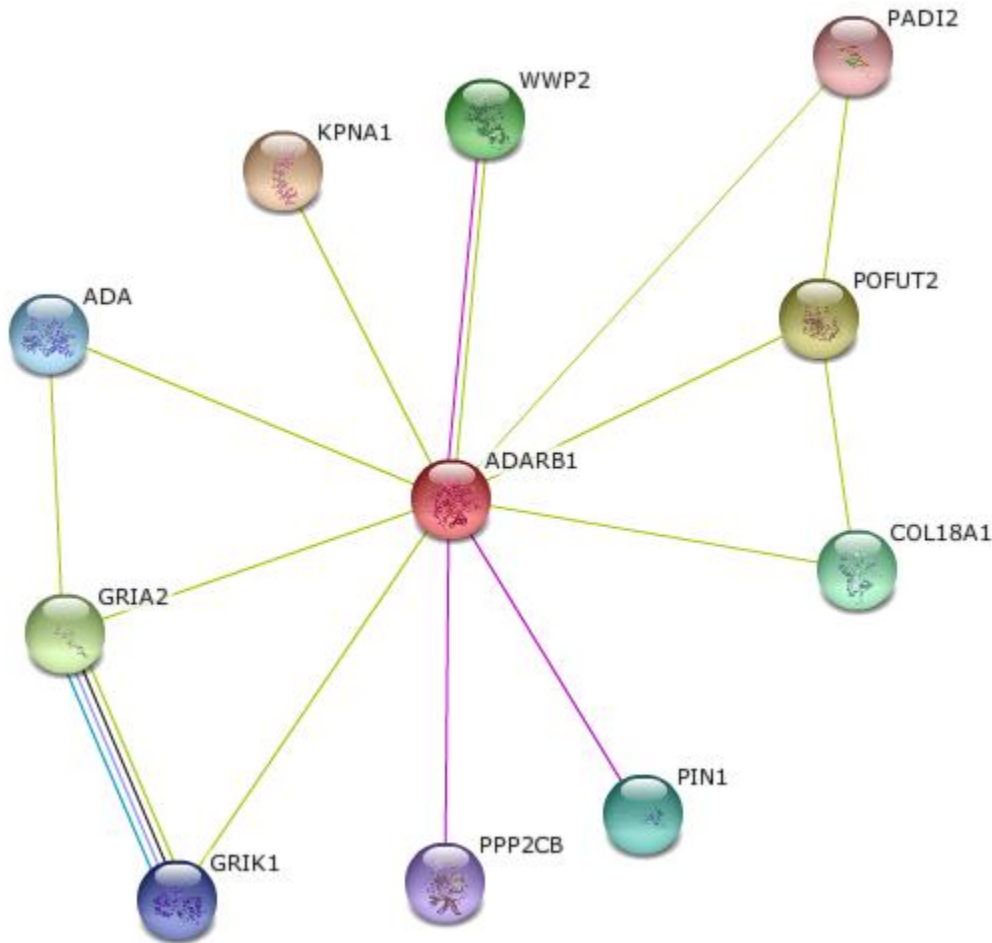
- WWP2: NEDD4-like E3 ubiquitin-protein ligase WWP2

- PPP2CB:Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform

- PIN2:Telomeric repeat-binding factor 1

大部分基于序列预测，缺乏实验证据的支持。

Homology Modeling of ADAR2

Search ADAR2 in PDB database

| 2B7T	2B7V	2L3C	2L2K	2L3J	1ZY7
Structure of ADAR2 dsRBM1	Structure of ADAR2 dsRBM2	Solution structure of ADAR2 dsRBM1 bound to LSL RNA	Solution NMR structure of the R/G STEM LOOP RNA-ADAR2 DSRBM2 Complex	The solution structure of the ADAR2 dsRBM-RNA complex reveals a sequence-specific read out of the minor groove	Crystal structure of the catalytic domain of an adenosine deaminase that acts on RNA (hADAR2) bound to inositol hexakisphosphate (IHP)
NMR					X-RAY

2B7T
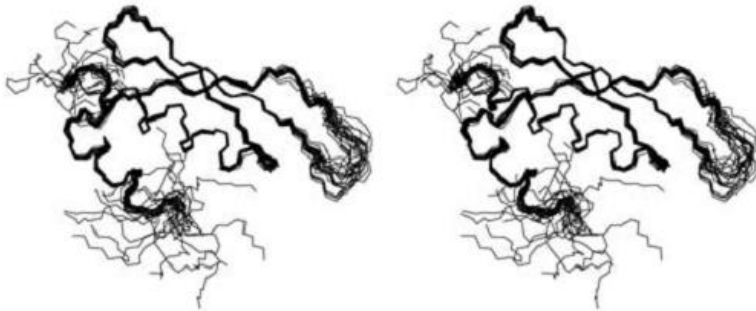
DRBM1
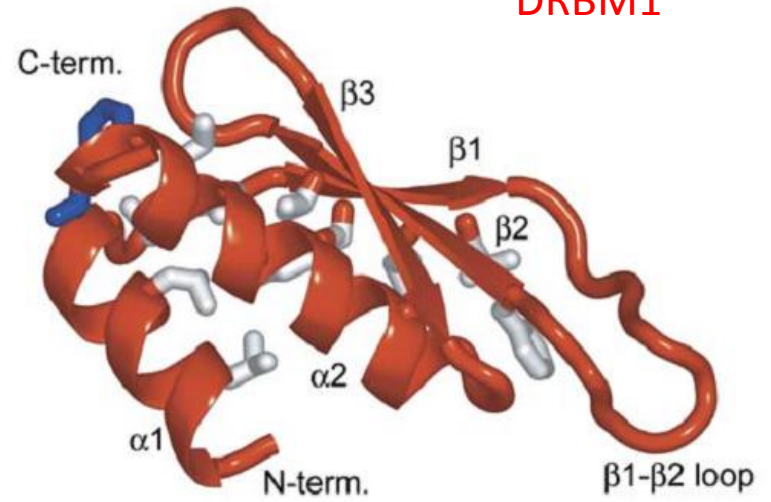
2B7V
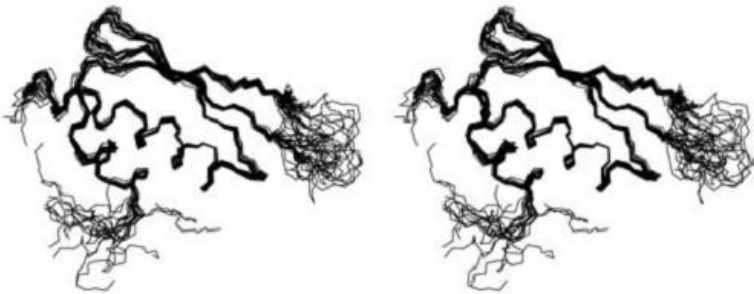
DRBM2
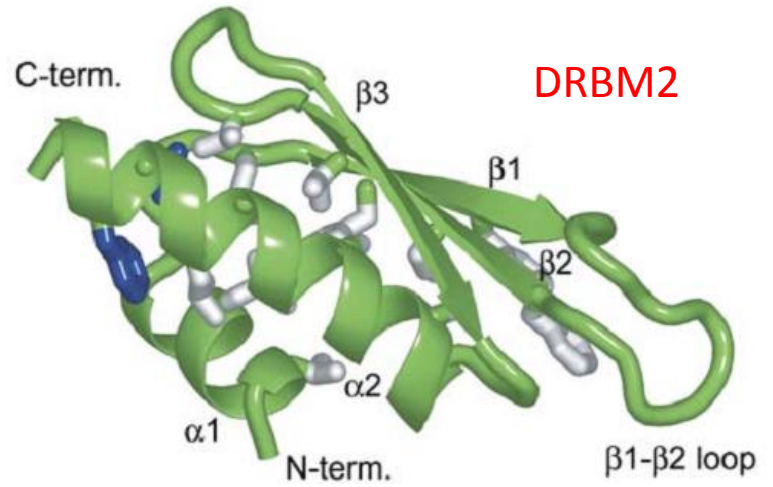
Structure. 2006 Feb;14(2):345-55.

2L3C

2L3C

2L3k

Cell. 2010 Oct 15;143(2):225-37.
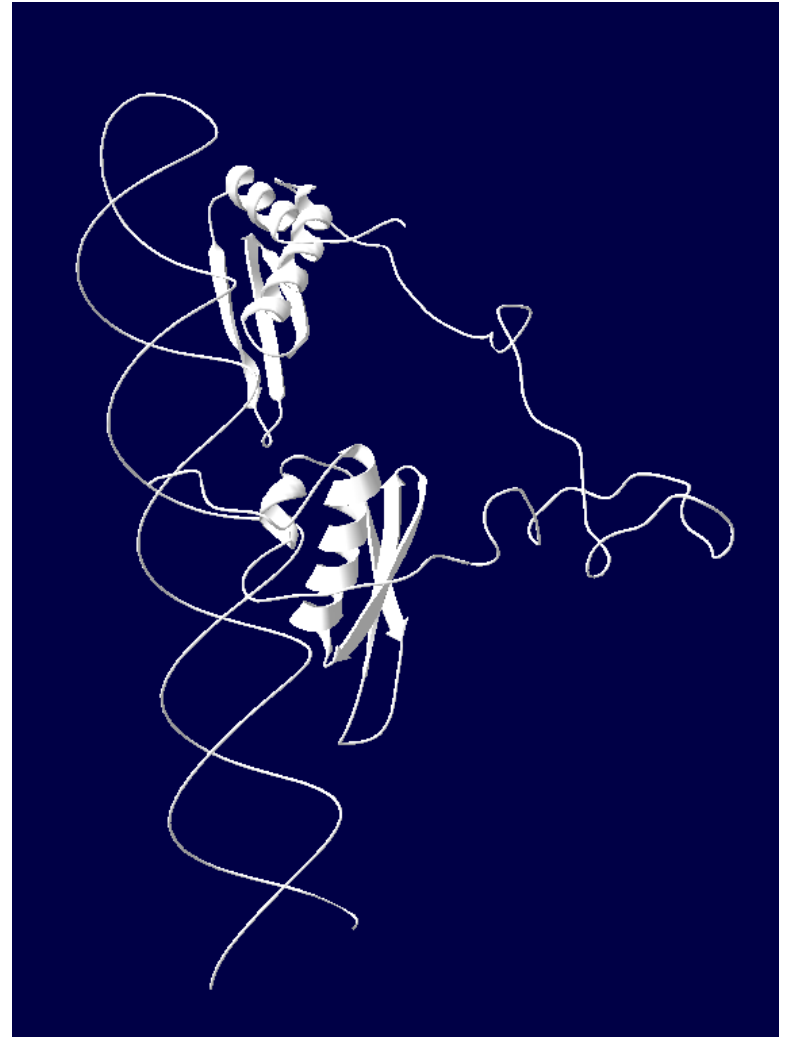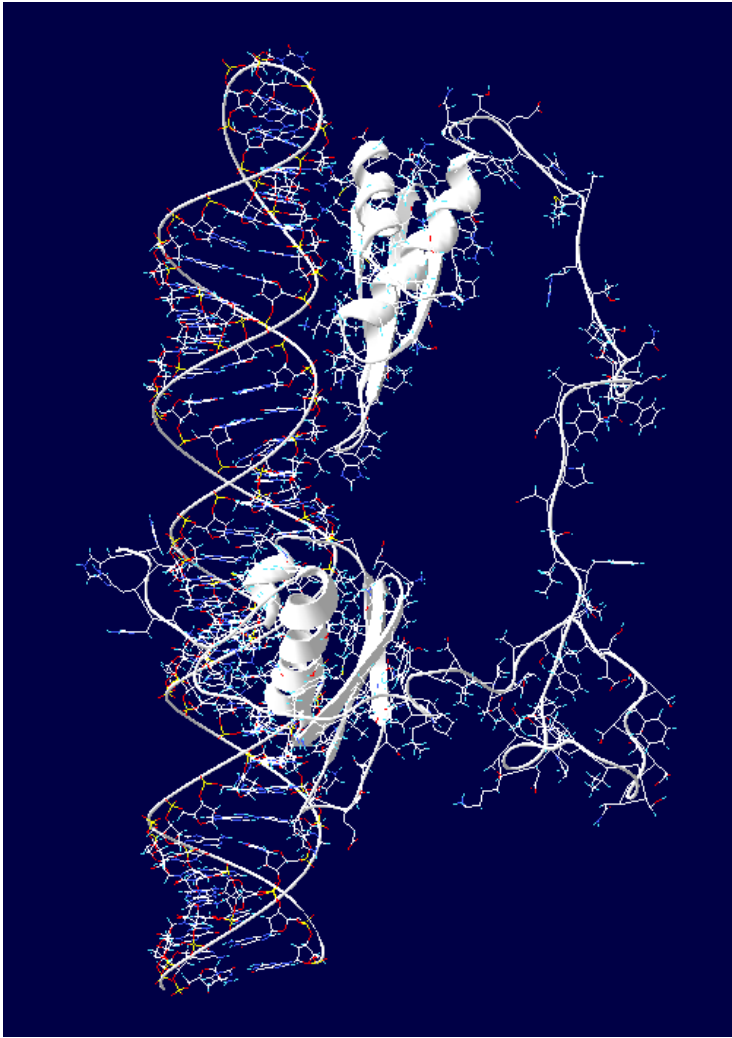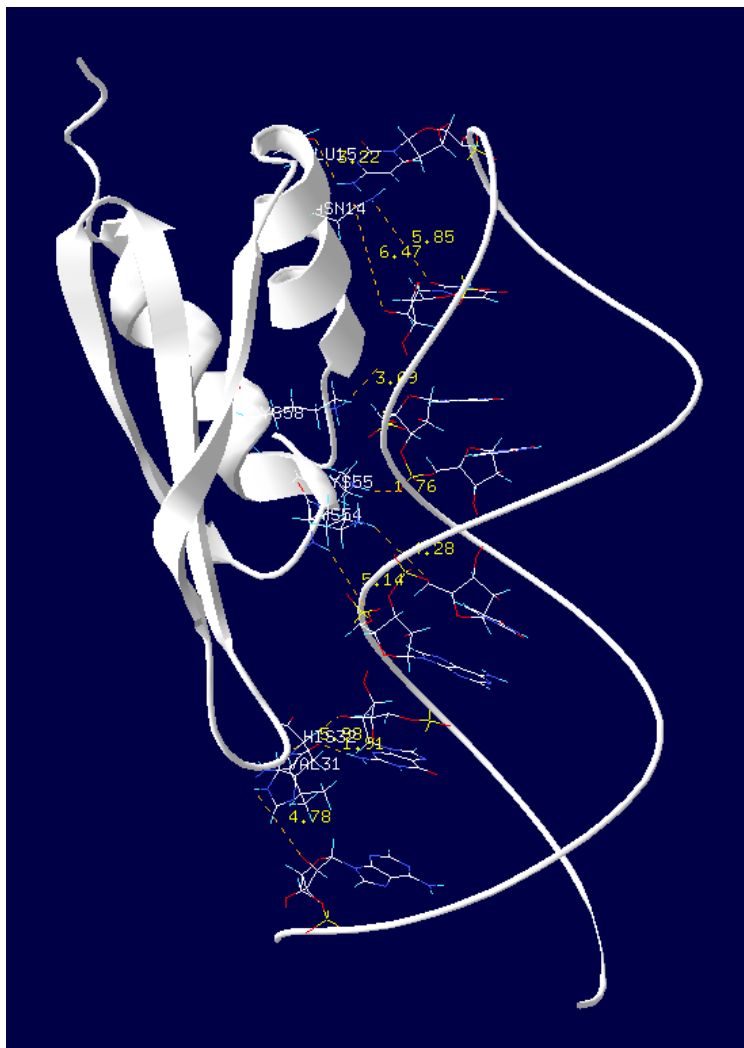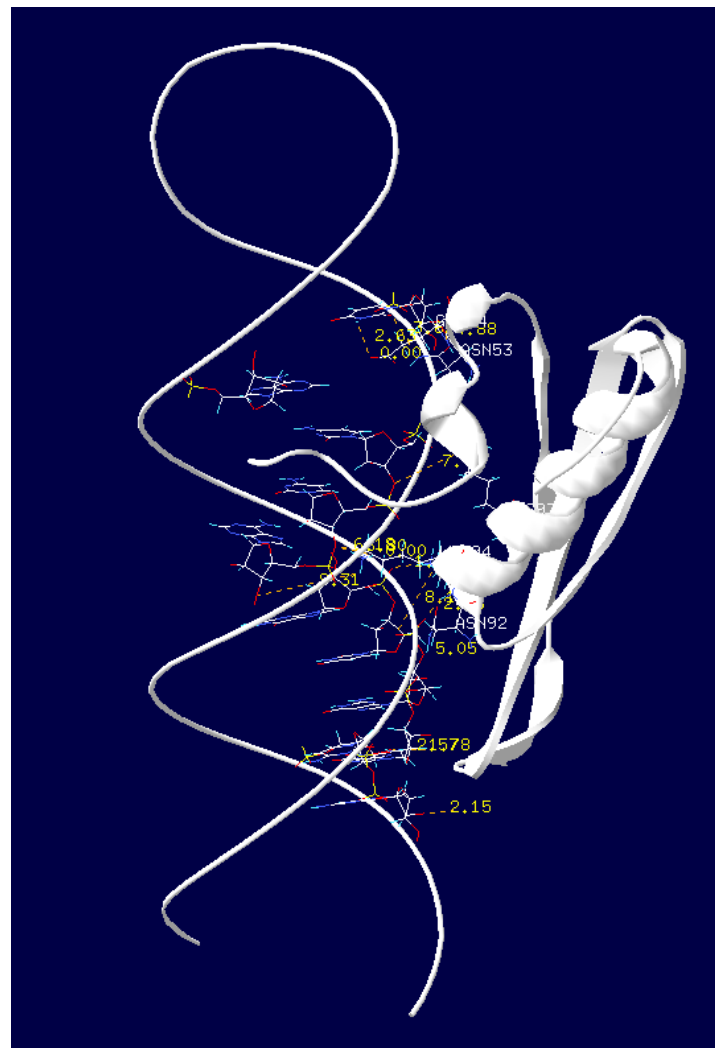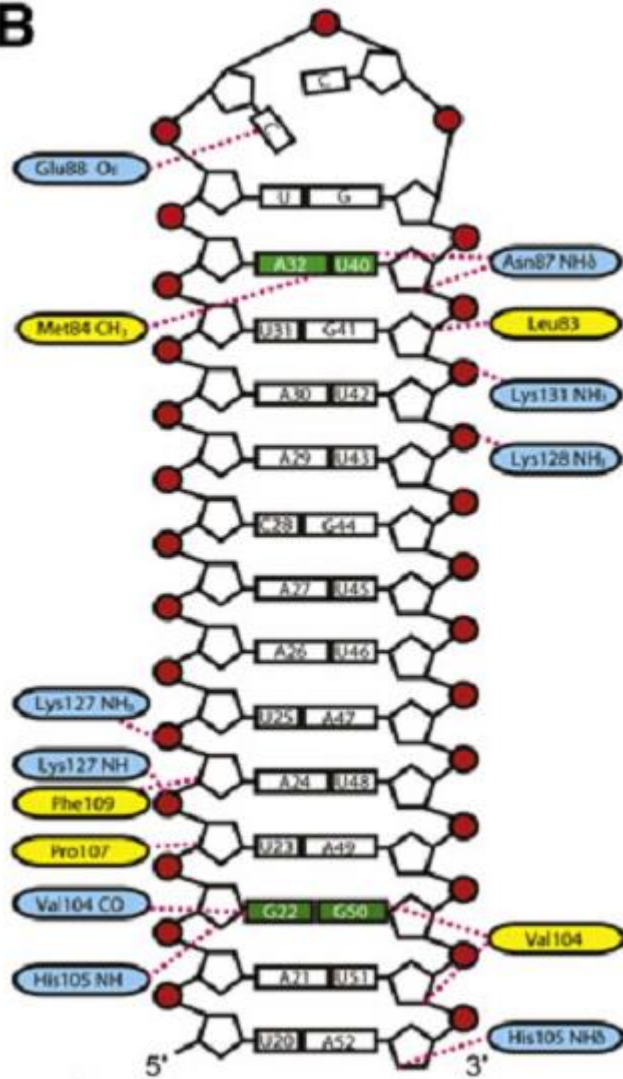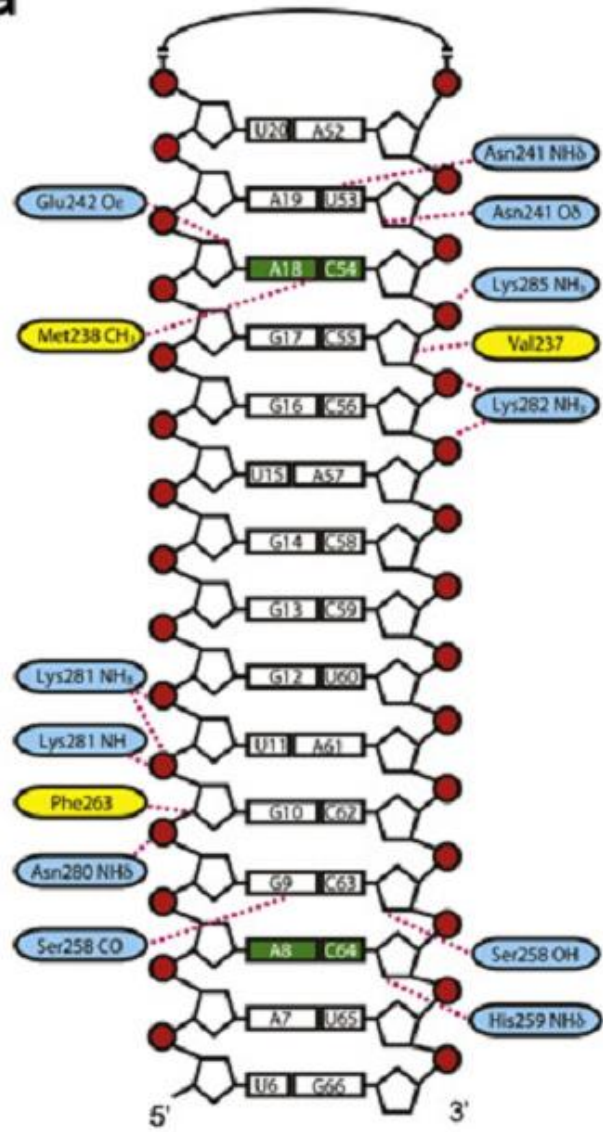
Pre-microRNA from miRBase

```
>hsa-mir-3180-2 MI0014215 Homo sapiens miR-3180-2 stem-loop
GCGACGGGCGGAGCUUCCAGACGCUCCGCCCCACGUCGCAUGCGCCCCGGGAAAGCGUGG
>hsa-mir-6875 MI0022722 Homo sapiens miR-6875 stem-loop
GAGUCUGAGGGACCCAGGACAGGAGAAGGCCUAUGGUGAUUUGCAUUCUUCCUGCCCUGG
test.txt  (END)
```

Homo sapiens miR-6875 stem-loop

```
gagu       ---  c       a      -   g  cu  g
    cugagg     ga ccagg cagga gaa gc  au g
    ||||||     || ||||| ||||| ||| ||  ||
    gacucc     cu ggucc guccu cuu cg  ua u
----       uac  c       c      u   a  uu  g
```

Homo sapiens miR-3180-2 stem-loop

```
gcgac       a       a  c              ----------   c
    gggcgg gcuucc ga gcuccgccccacgu            cg a
    |||||| |||||| || ||||||||||||||            ||
    cccgcc cggagg cu cgaggcggggugcg            gc u
--cgu       c       c  u              aaagggcccc   g
```

http://www.mirbase.org/

microRNA Structure Prediction
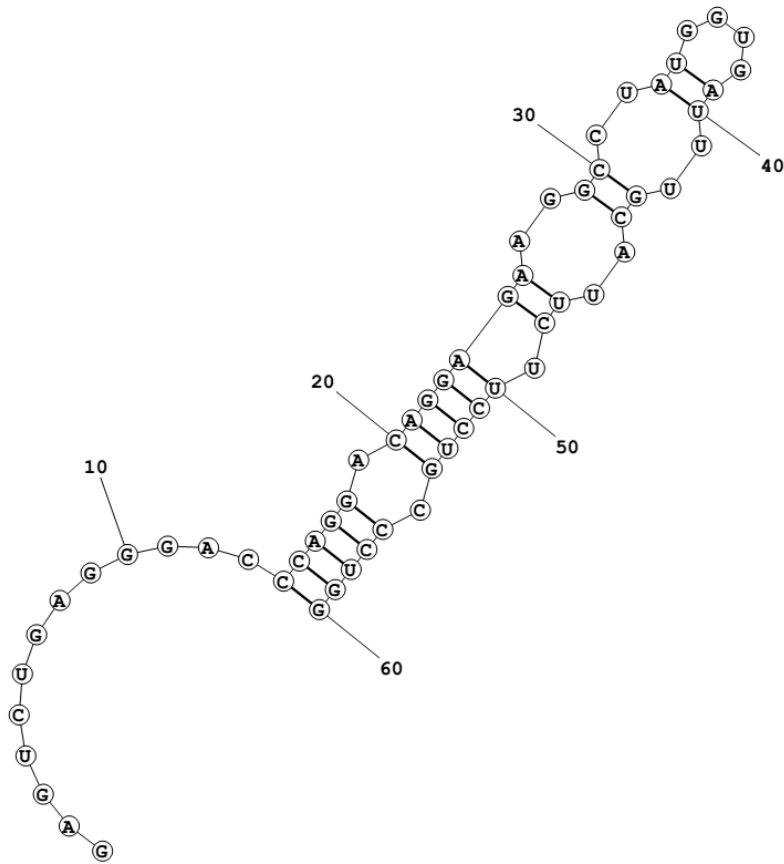
ENERGY = 5.3 hsa-mir-6875 MI0022722...
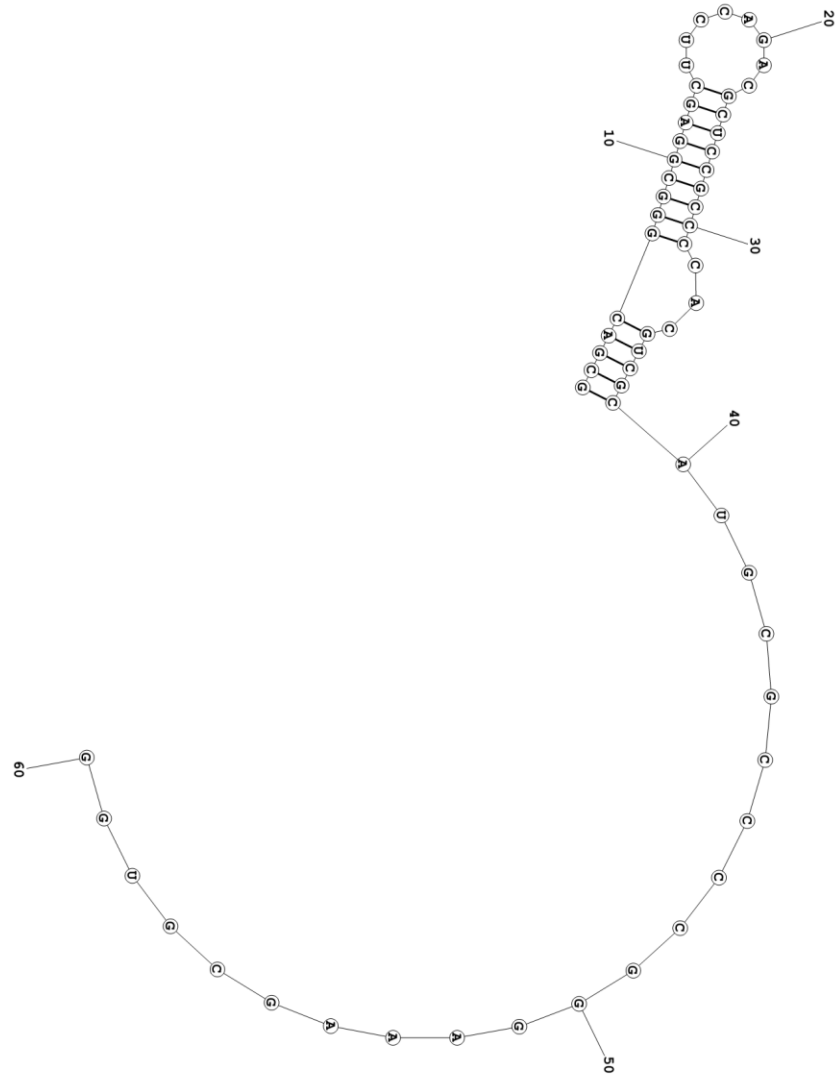
ENERGY = 5.7 hsa-mir-3180-2 MI00142...

http://rna.urmc.rochester.edu/RNAstructure.html

小结与讨论

- ADARB1是一类普遍存在的RNA编辑酶，在物种间的氨基酸序列高度保守。

- 从结构上看，ADARB1上的DRBM符合RNA binding protein 的DRBM的普遍结构特征，由αββα的二级结构组成，但是除了几个比较保守的位点之外，不同的DRBM的氨基酸序列相似性并不高。

- 通过核磁共振对ADARB1的两个DRBM结合dsRNA的结构解析，我们了解到DRBM中的α1-helix 和β1-β2 loop是结合RNA的重要部分，它的结合具有一定的序列特异性。

- 根据文献提供的ADARB1结合的RNA序列特征，我们找到ADARB1可能的新的microRNA结合底物（hsa-miR-6875, hsa-miR-3180-2），并预测了它们的RNA二级结构，但仍需进一步三维建模以及实验数据验证（如CLIP-Seq等）。

参考文献

1. Many roads to maturity: microRNA biogenesis pathways and their regulation. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Nat Cell Biol. 2009 Mar;11(3):228-34. doi: 10.1038/ncb0309-228.

2. MicroRNA: basic mechanisms and transcriptional regulatory networks for cell fate determination. Fazi F, Nervi C. Cardiovasc Res. 2008 Sep 1;79(4):553-61. doi: 10.1093/cvr/cvn151.

3. The many roles of an RNA editor. Keegan LP, Gallo A, O'Connell MA. Nat Rev Genet. 2001 Nov;2(11):869-78.

4. RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Higuchi M, Single FN, Köhler M, Sommer B, Sprengel R, Seeburg PH. Cell. 1993 Dec 31;75(7):1361-70.

5. Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. Yang W, Chendrimada TP, Wang Q, Higuchi M, Seeburg PH, Shiekhattar R, Nishikura K. Nat Struct Mol Biol. 2006 Jan;13(1):13-21.

6. Structure and specific RNA binding of ADAR2 double-stranded RNA binding motifs. Stefl R, Xu M, Skrisovska L, Emeson RB, Allain FH. Structure. 2006 Feb;14(2):345-55.

7. The solution structure of the ADAR2 dsRBM-RNA complex reveals a sequence-specific readout of the minor groove. Stefl R, Oberstrass FC, Hood JL, Jourdan M, Zimmermann M, Skrisovska L, Maris C, Peng L, Hofr C, Emeson RB, Allain FH. Cell. 2010 Oct 15;143(2):225-37. doi: 10.1016/j.cell.2010.09.026.

Thank you!