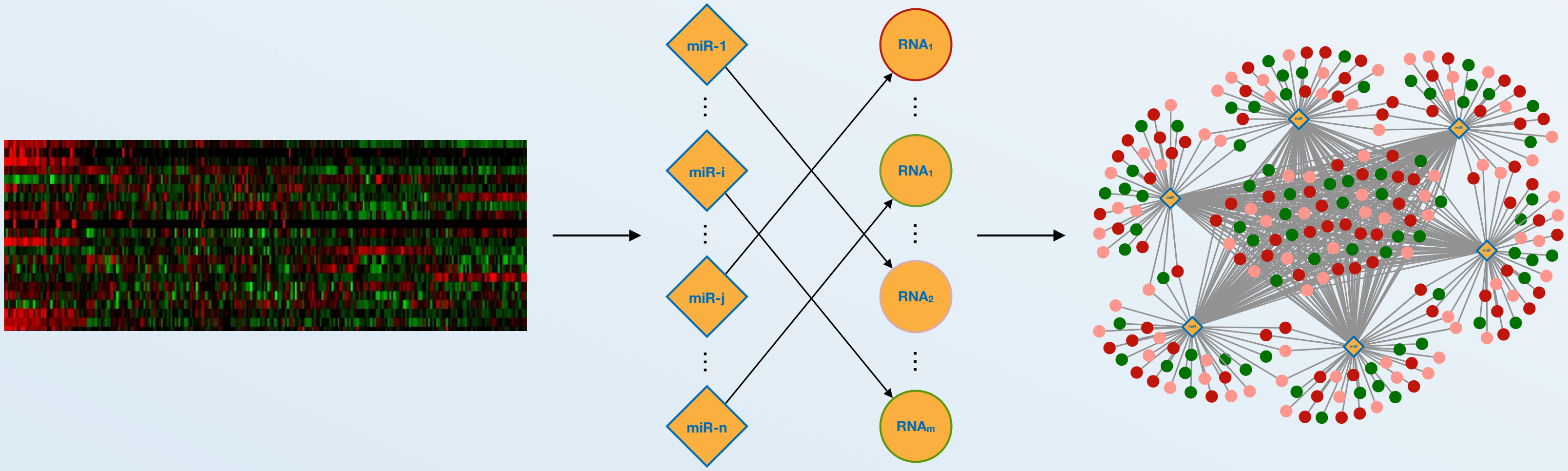
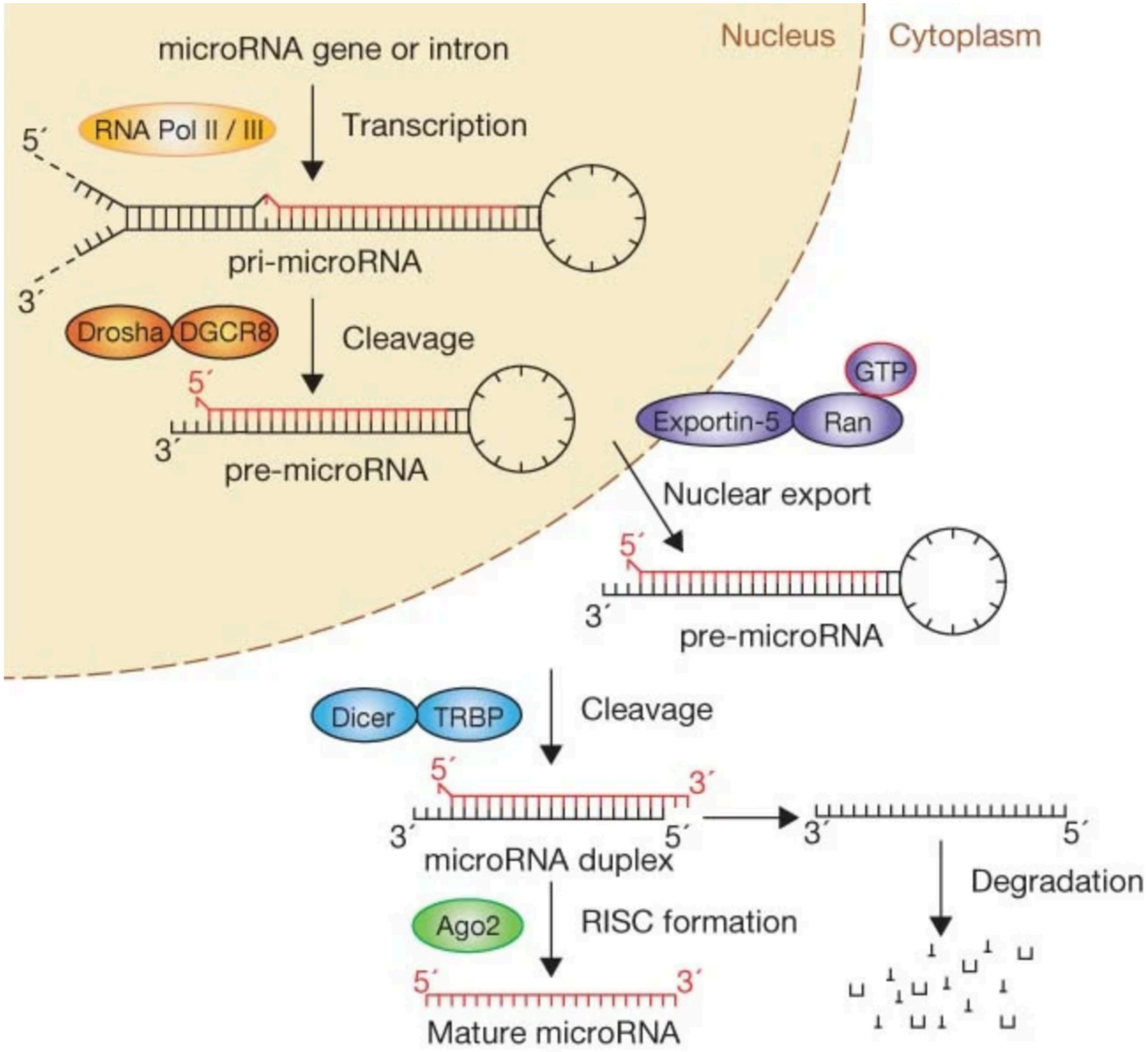
RNA Engineering Laboratory

Dancing to Silence

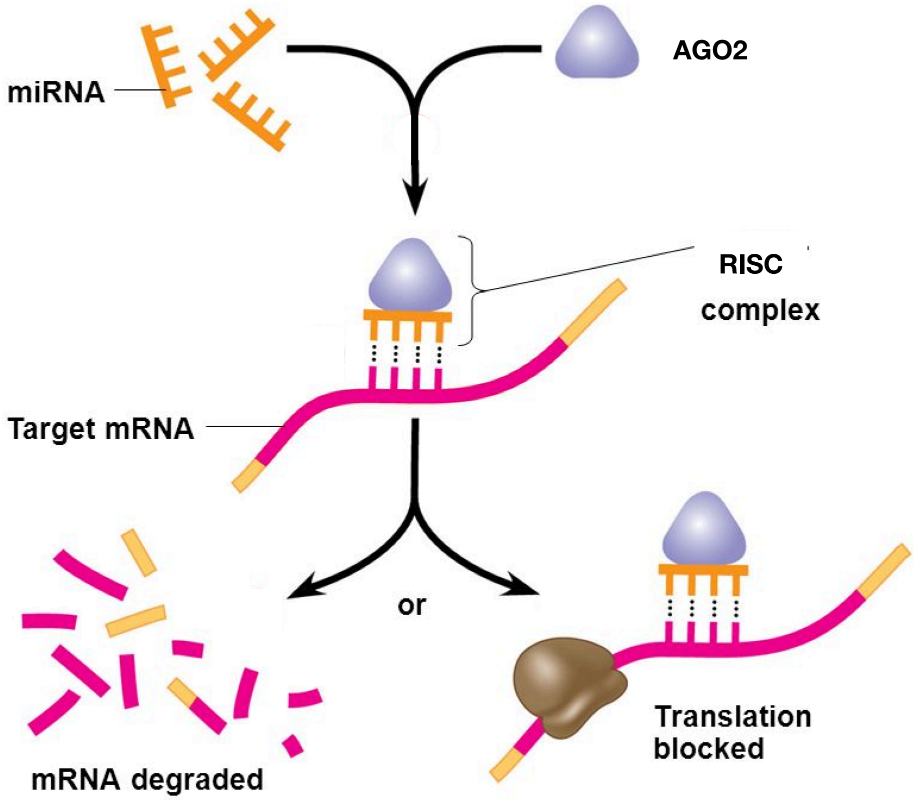


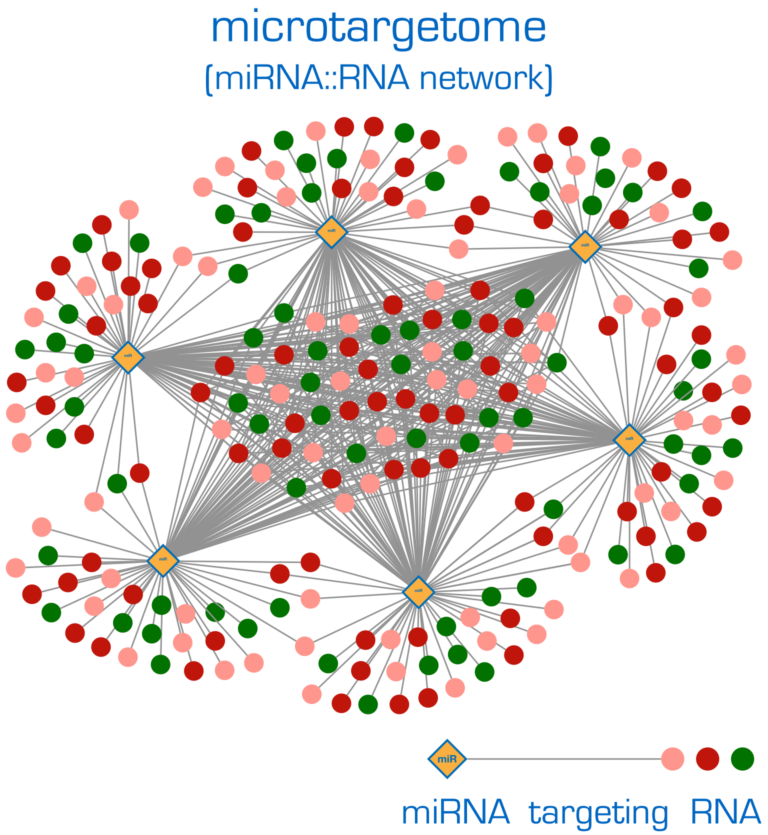
There are multiple RBPs on the way to producing a mature microRNA



Winter et al. 2009 *Nat Cell Biol*

MicroRNAs are important regulators of gene expression that control both physiological and pathological processes such as development and cancer



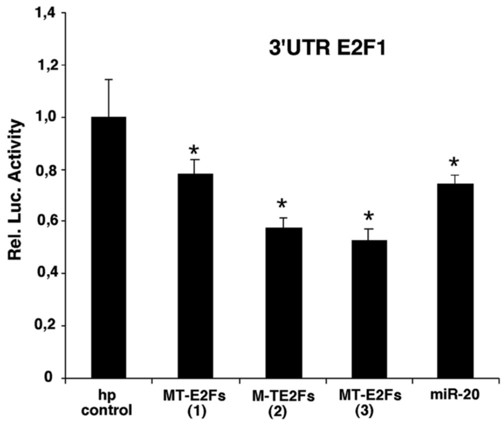


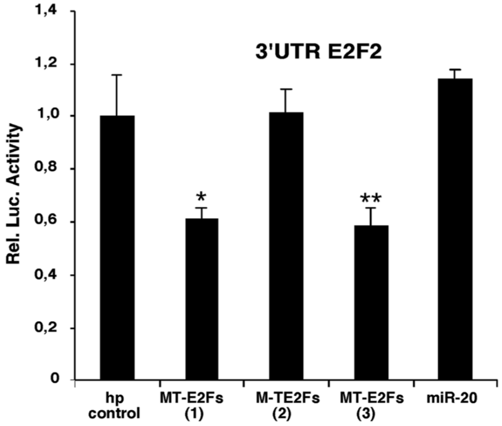
- MicroRNAs participate in most aspects of cellular differentiation and homeostasis, and consequently have roles in many pathologies, including cancer.;
- MicroRNAs exert their effects in the context of complex regulatory networks (microtargetomes), made all the more extensive by the inclusion of transcription factors as their direct targets.

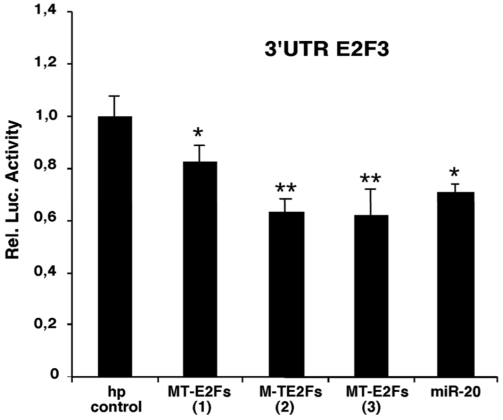
Bracken et al. 2016 *Nat Rev Genetics*

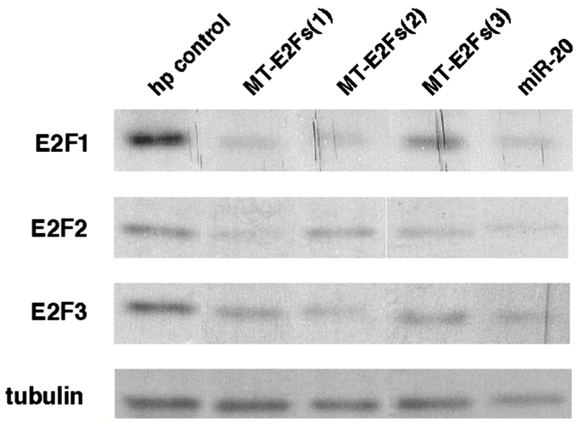
Designing small multiple-target artificial RNAs

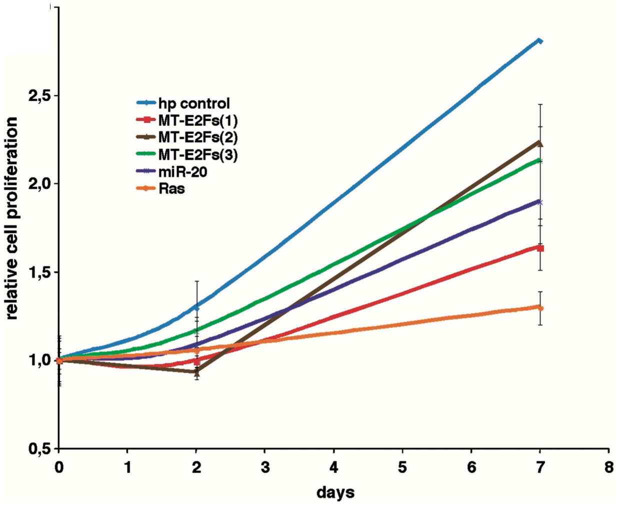
		E2F1	E2F2	E2F3
miR-20	UAAAGUGCUUAUAGUGCAGGUAG	363–395 956–988	881–913 1495–1527 3259–3291	1800–1832
MT-E2Fs(1)	UAUCUGACUUACGUGACUGCUU	740–772	974–1006	1188–1220
MT-E2Fs(2)	UUUCCCAAUUUCGCCCGGCCCU	707–739	2019–2051	1838–1870
MT-E2Fs(3)	UAGUGGGGAGGGGGUUUCCGGU	93–125 946–978	2262–2294 2734–2766	709–741 1219–1251





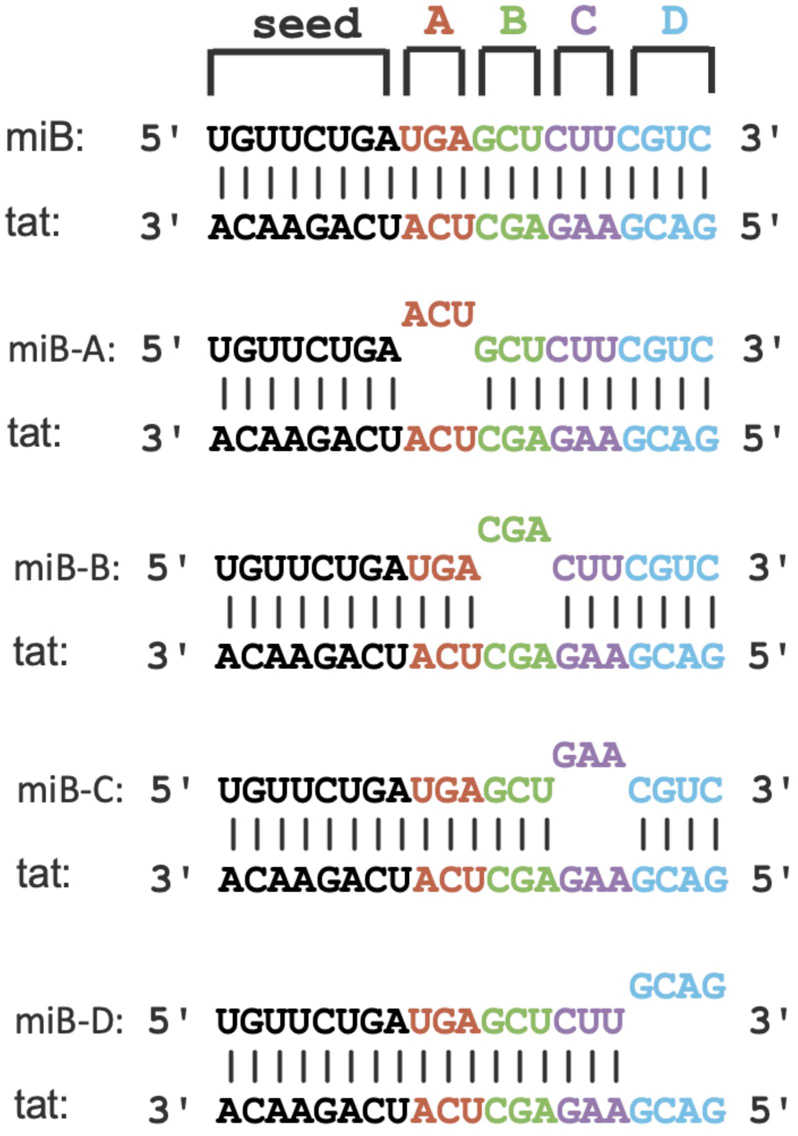




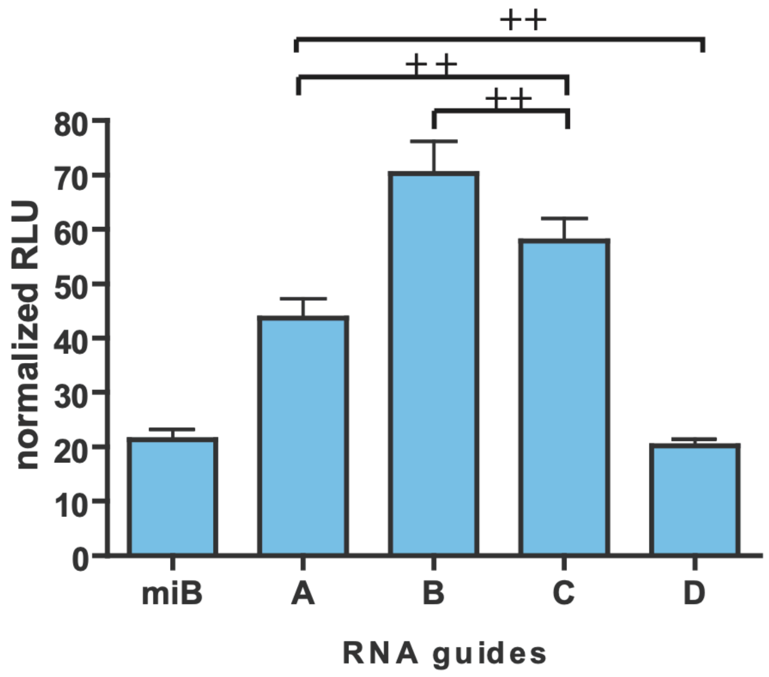


De Guire et al. 2010 *NAR*

The sequence features that define efficient and specific hAGO2-dependent miRNA silencing guides



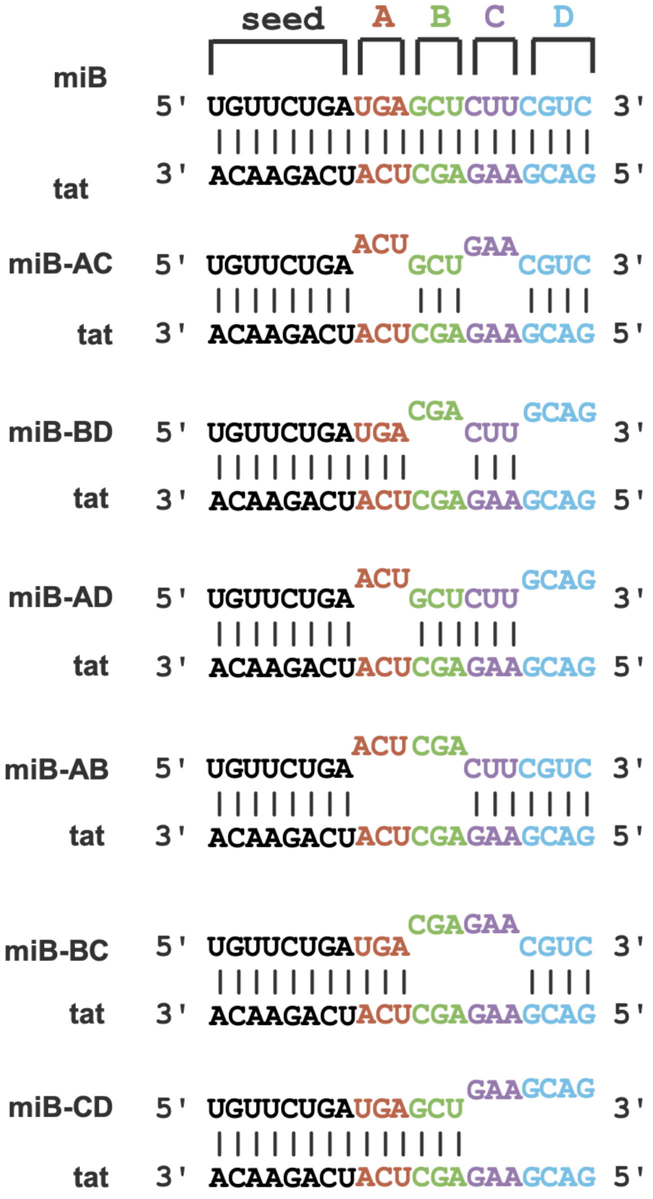
```
           seed        A  B  C   D
miB:    5' UGUUCUGAUGAGCUCUUCGUC 3'
           |||||||||||||||||||||
tat:    3' ACAAGACUACUCGAGAAGCAG 5'

                   ACU
miB-A:  5' UGUUCUGA   GCUCUUCGUC 3'
           ||||||||   ||||||||||
tat:    3' ACAAGACUACUCGAGAAGCAG 5'

                      CGA
miB-B:  5' UGUUCUGAUGA   CUUCGUC 3'
           |||||||||||   |||||||
tat:    3' ACAAGACUACUCGAGAAGCAG 5'

                         GAA
miB-C:  5' UGUUCUGAUGAGCU   CGUC 3'
           ||||||||||||||   ||||
tat:    3' ACAAGACUACUCGAGAAGCAG 5'

                            GCAG
miB-D:  5' UGUUCUGAUGAGCUCUU     3'
           |||||||||||||||||
tat:    3' ACAAGACUACUCGAGAAGCAG 5'
```

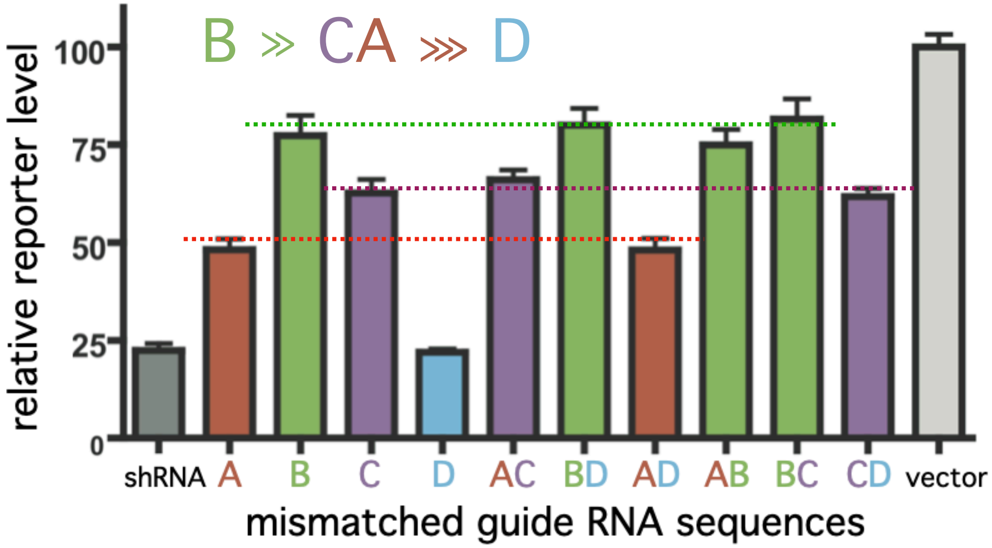


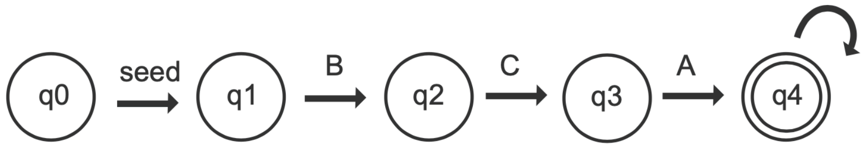
100 > B > C > A > D ≃ 0

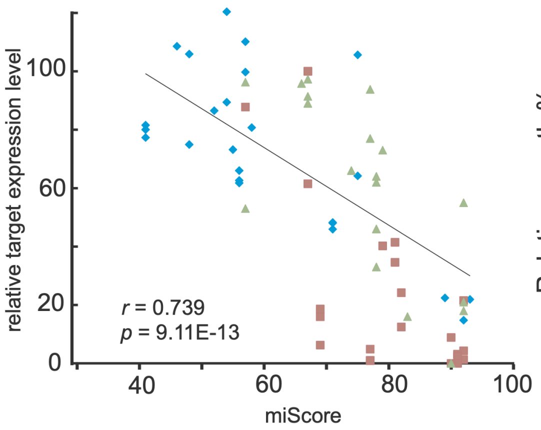
Yan et al. 2018 *NAR*

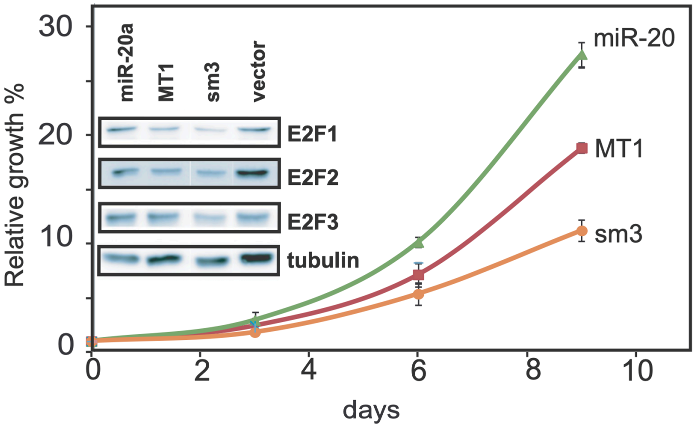
AGO2 acts as a deterministic finite automaton







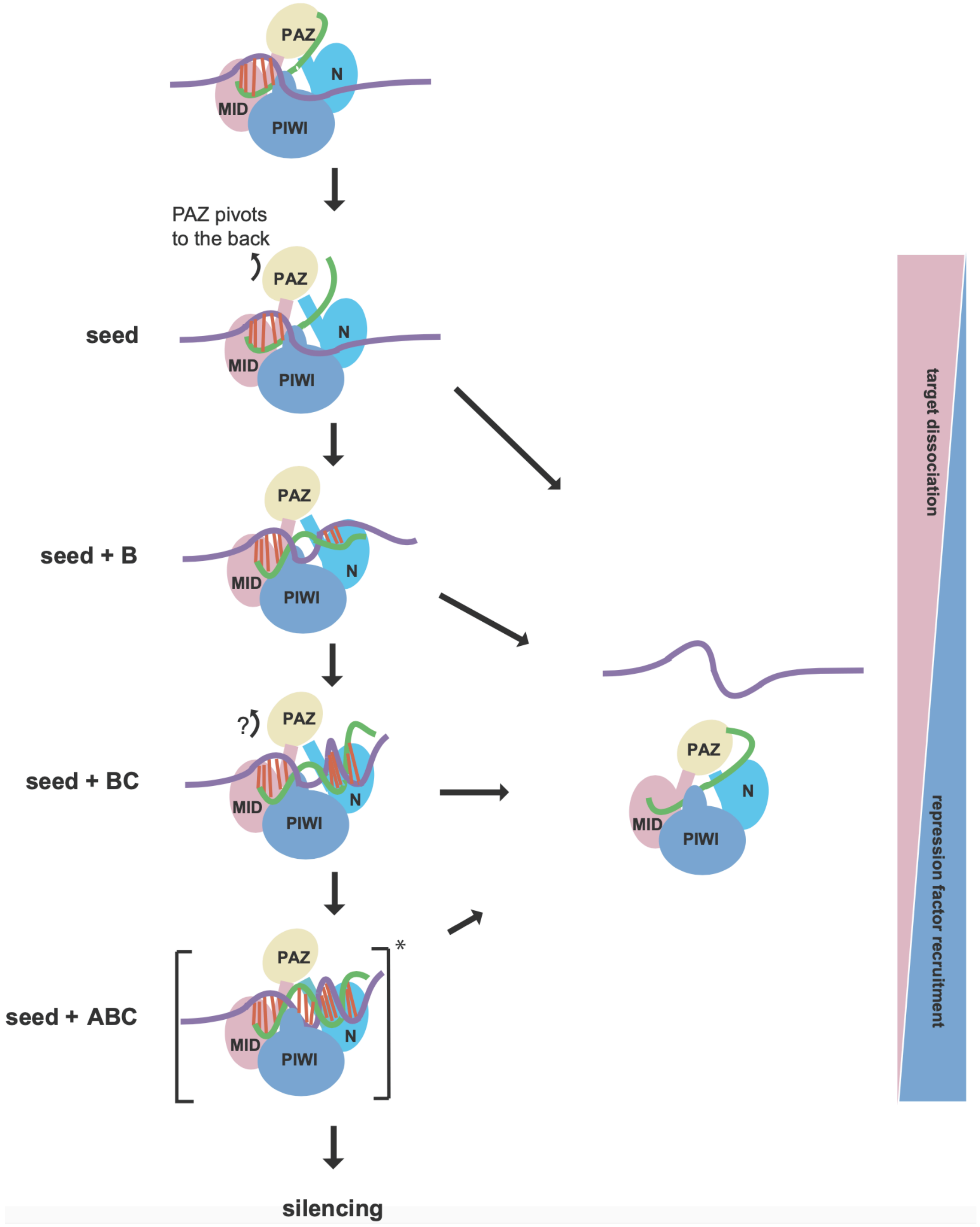




Wee et al. 2012 *Cell* (Zamore)
Robertson et al. 2010 *Silence* (Dharmacon)

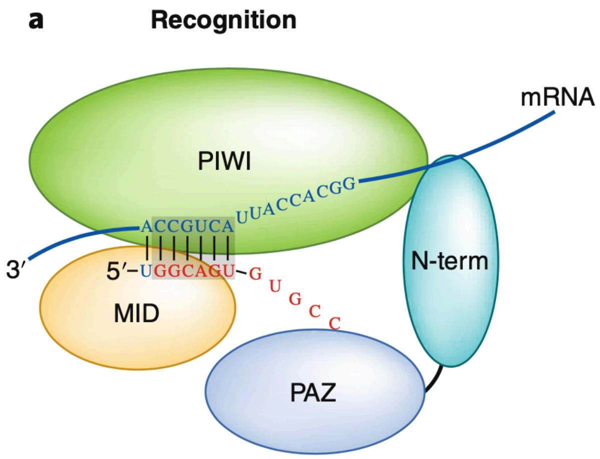
Yan et al. 2018 *NAR*

Skipped-propagation and coordinated annealing model



Yan et al. 2018 *NAR*

The guide (micro) RNA is bound to the MID and PAZ domains; nucleotides 2 to 5 or 6 are exposed



Baronti et al. 2020 *Nature*
Major 2020 *NSMB*

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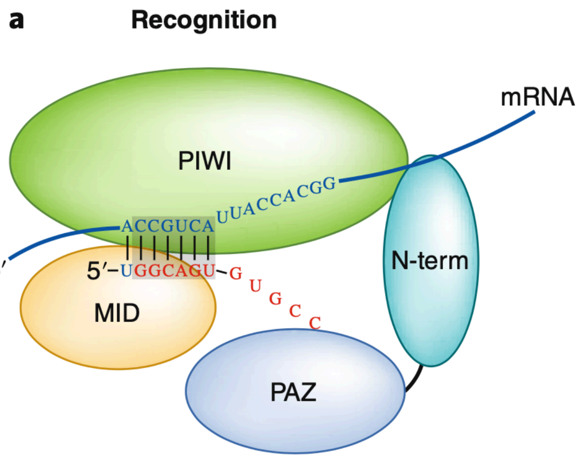

a. **Recognition** occurs through WC base pairs

Baronti et al. 2020 *Nature*
Major 2020 *NSMB*

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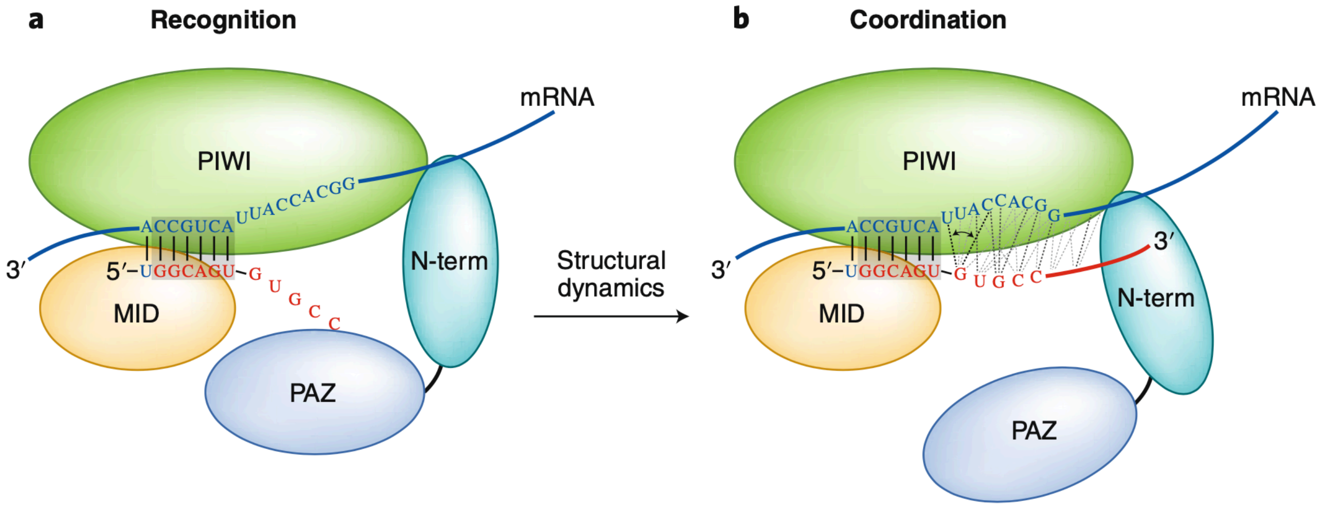

a. **Recognition** occurs through WC base pairs

b. **Coordination** involves transient states where supplementary base pairs extend and stabilize the RNA duplex, leading to the release of the microRNA 3’ end from the PAZ domain and opening of the PAZ and N-terminal domains

Baronti et al. 2020 *Nature*
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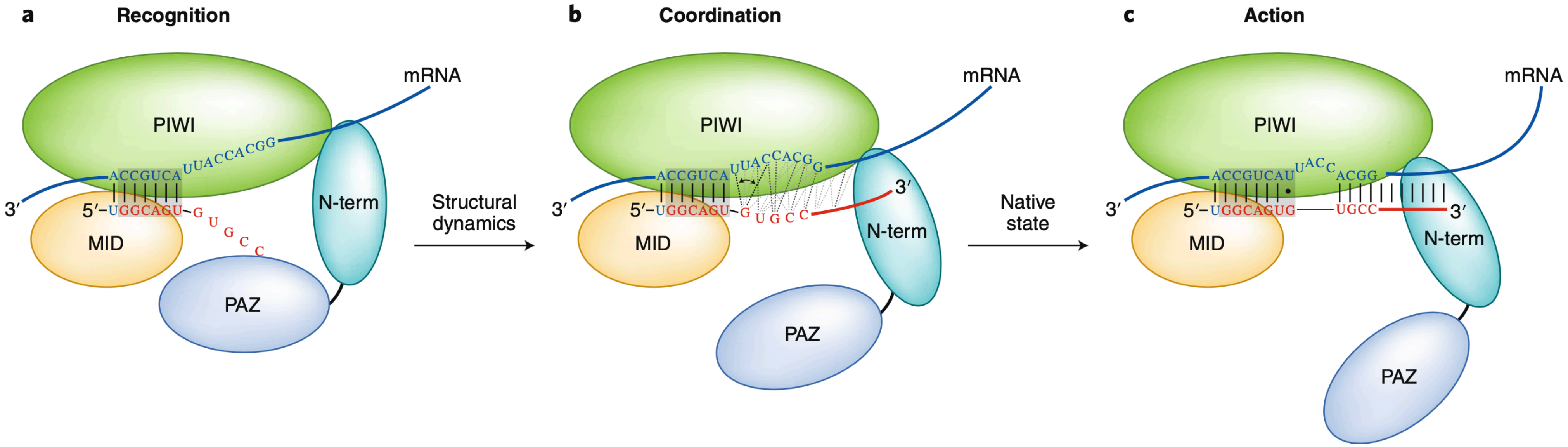


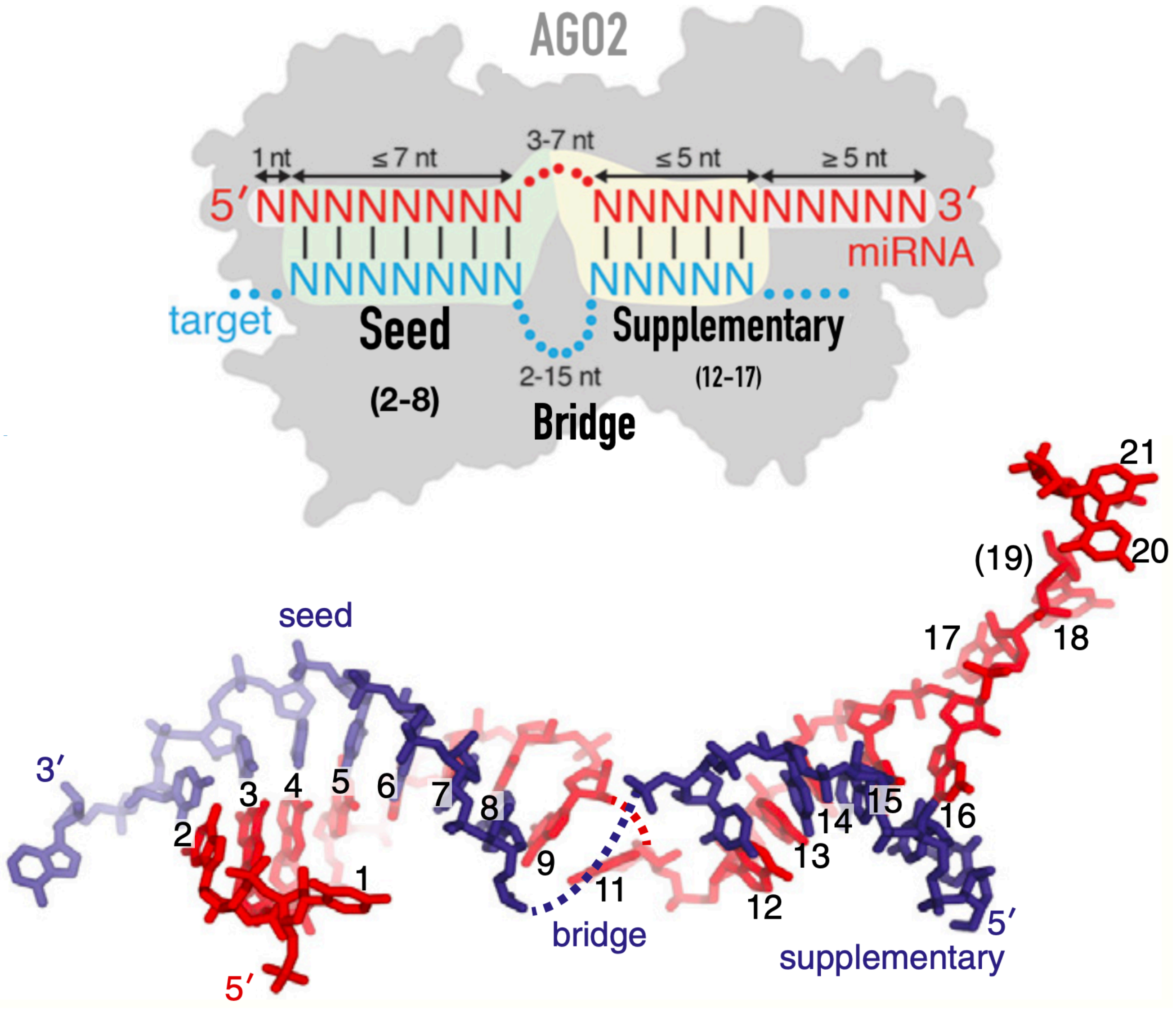
a. **Recognition** occurs through WC base pairs

b. **Coordination** involves transient states where supplementary base pairs extend and stabilize the RNA duplex, leading to the release of the microRNA 3’ end from the PAZ domain and opening of the PAZ and N-terminal domains

c. **Action** degrades or destabilizes the target RNA with an efficiency that depends on the formation and structure of the duplex.

Baronti et al. 2020 *Nature*
Major 2020 *NSMB*

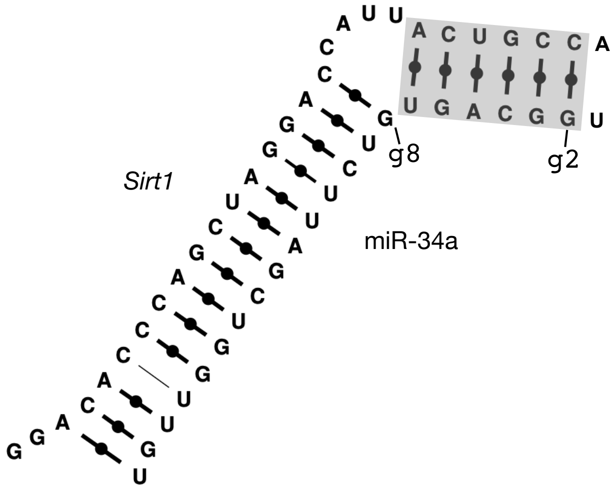
Seed and supplementary chambers are adjacent to each other and can be bridged by an unstructured target loop of up to 15 nucleotides



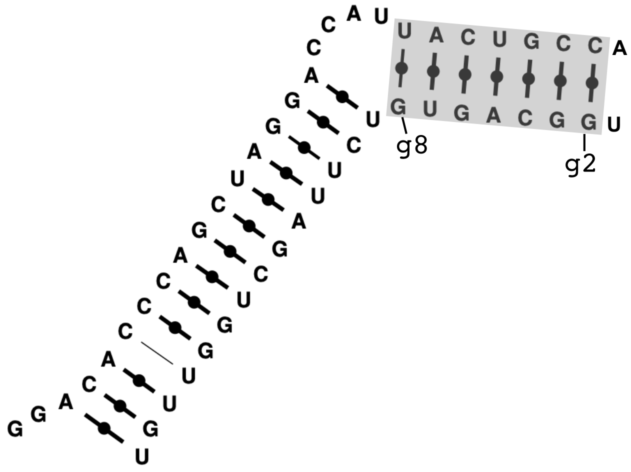
Sheu-Gruttadauria et al., 2019 *EMBO J*

MiR-34a targets Sirt1's 3'UTR in a tumor suppressive feedback loop
Sirt1 (silent information regulator 1) is a p53 deacetylation enzyme

```
mcff -s GGACACCCAGCUAGGACCAUUACUGCC -sd GGCAGUGUCUUAGCUGGUUGU -e 5 -v -alt
..(((<(((((((<(((....((((((    )))))))))>)))))))>))) -54.002 (7-mer-A1)
<..((<(((((((<(((....((((((    )))))))))>)))))))>))> -52.270 (7-mer-A1)
..(((<(((((((<((....<((((((    ))))))>))>)))))))>))) -52.237 (8-mer)
.<.((<(((((((<(((....((((((    )))))))))>)))))))>))> -52.189 (7-mer-A1)
..(((.(((((((<(((....((((((    )))))))))>))))))).))) -51.875 (7-mer-A1)
..(((<(((((((<(((<....(((((    )))))>)))>)))))))>))) -51.604 (none)
GGACACCCAGCUAGGACCAUUACUGCCA  UGGCAGUGUCUUAGCUGGUUGU
Sirt1                        g12345678               miR-34a
```



Minimum Free Energy (MFE)
7-mer-A1



Suboptimal state (+1.77 kcal/mol)
8-mer

- Recognition occurs through six WC base pairs (**7-mer-A1** seed type) (left);
- Transient state with additional G:U base pair forms a more stable **8-mer** seed type (right).

Baronti et al. 2020 *Nature*

A scan of all mRNAs in 113,982 transcripts (NCBI RefSeq) revealed 30,721 miR-34a dynamic seeds (27%)

```
5'     ...CCAGCUAGGACCAUUACUGCCAG... Sirt1
           ||||||        ||||||
3'    CGUUAGUCGAUUGAUG---UGACGGA     miR-34a
```

7-mer-A1 Targetscan

```
5'-ggacacccagcuaggaccauuacugccA-3' Sirt1
     |||:|||||||!|||    ||||||
3'---uguugGUCGAUucuG----UGACGGU-5' miR-34a
```

7-mer-A1 `mcff` MFE

```
5'-ggacacccagcuaggaccauuacugccA-3' Sirt1
     |||:|||||||!||    !||||||
3'---uguugGUCGAUucu----GUGACGGU-5' miR-34a
```

8-mer `mcff` +1.77 kcal/mol

- First-in-man Phase I study of a miRNA-based cancer therapy was with a miRNA-34a-5p mimic
- The miR-34a-5p mimic was absorbed by tumor cells and immune cells
- Terminated due to serious immune system-related adverse events => must unravel effects on tumor and immune cells.
- Overexpression of miR-34a-5p in M1 macrophages leads to reduced secretion of chemokines CXCL10 and CXCL11
- In CD4+ and CD8+ T cells to a reduced expression of CXCR3 => major decrease in lymphocytes and other immune cells
- MiR-34a-5p targets include one dynamic site in the 3'UTR of CXCL10

```
5'-augcucuuuacuucauggacuuccacugccA-3' CXCL10
   |  |::!!::||  | !|||    ||||||
3'-u--guugGUCGA--U-ucug----UGACGGU-5' miR-34a
```

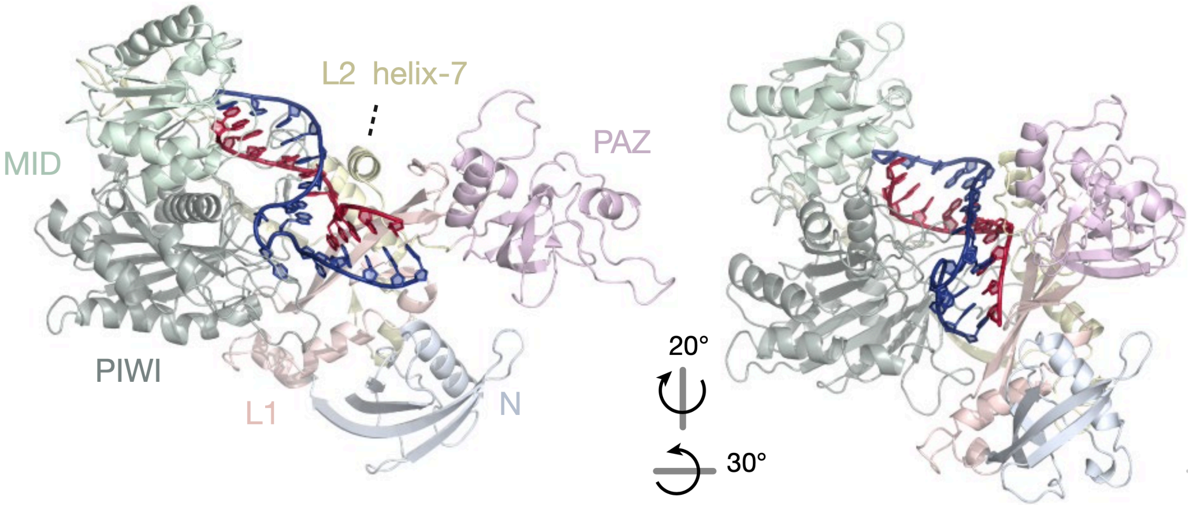
7-mer-A1 `mcff` MFE

```
5'-augcucuuuacuucauggacuuccacugccA-3' CXCL10
   |  |::!!::||  | !||    |||||||
3'-u--guugGUCGA--U-ucu----GUGACGGU-5' miR-34a
```

8-mer `mcff` +4.59 kcal/mol

Conclusions

- MiRNAs are fine-tuners of gene expression regulation and potentially powerful therapeutic agents
- MiRNAs naturally target multiple genes
- Smart RNAs use the inherent multi-targeting property of natural miRNAs
- The rules that define efficient and specific hAGO2-dependent miRNA silencing guides must be mastered to develop efficient miRNA-based therapeutics
- RNA structure dynamics play a role in RISC target recognition and action
- The motion of all parts of the miRNA:RNA duplex:hAGO2 complex is quite impressive, such as a perfect synchronized dance



Sheu-Gruttadauria et al., 2019 *EMBO J*

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CIHR IRSC Canadian Institutes of Health Research Instituts de recherche en santé du Canada

NSERC CRSNG

GenomeCanada

IRIC

Université de Montréal