‘irCLASH reveals RNA substrates recognized by human ADARs’

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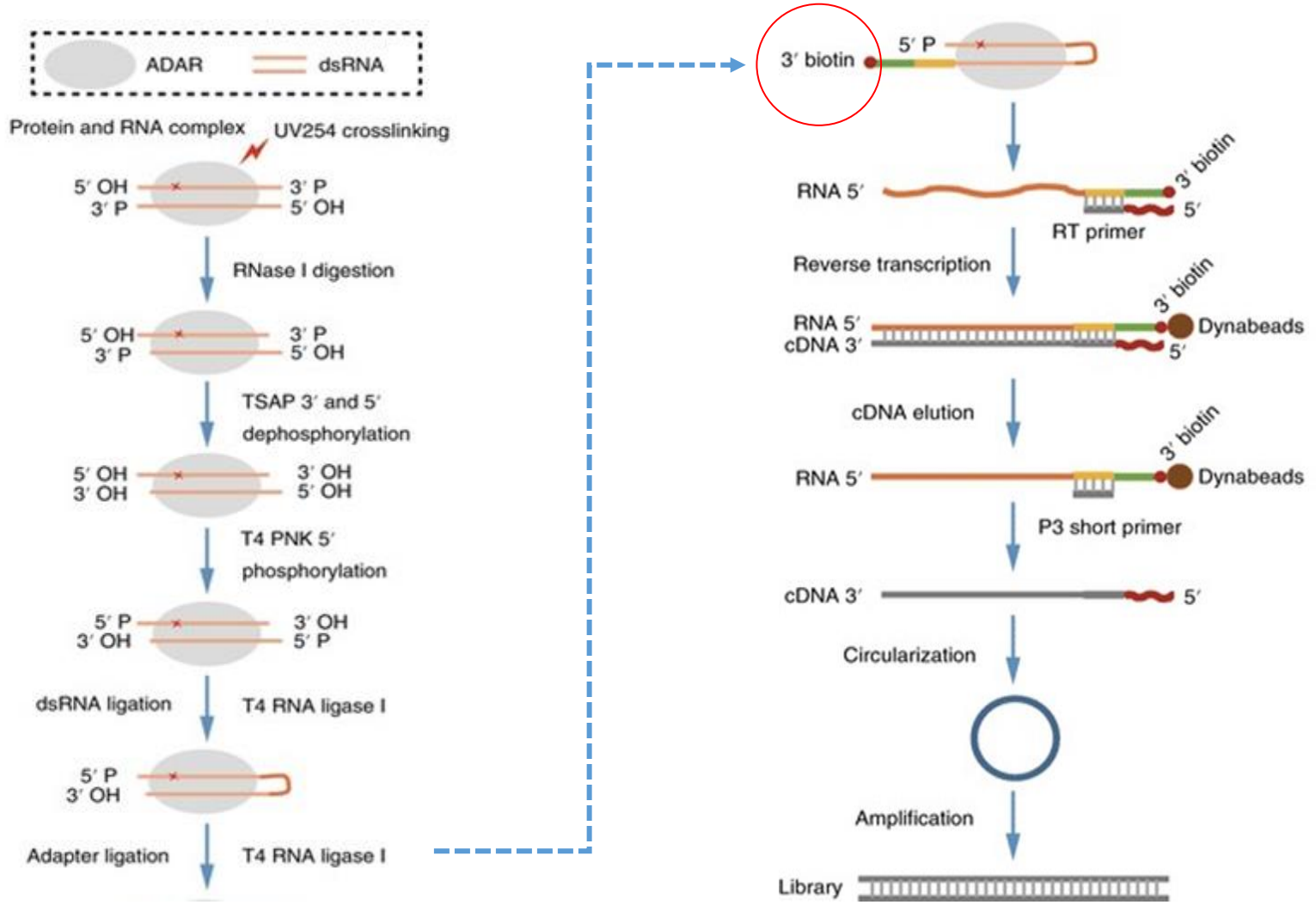
Schematic overview of the irCLASH method

- CLASH (crosslinking, ligation, and sequencing of hybrids)
- hiCLIP (RNA hybrid and individual-nucleotide resolution ultraviolet crosslinking and immunoprecipitation)
- irCLASH (infrared crosslinking, ligation and sequencing of hybrids)

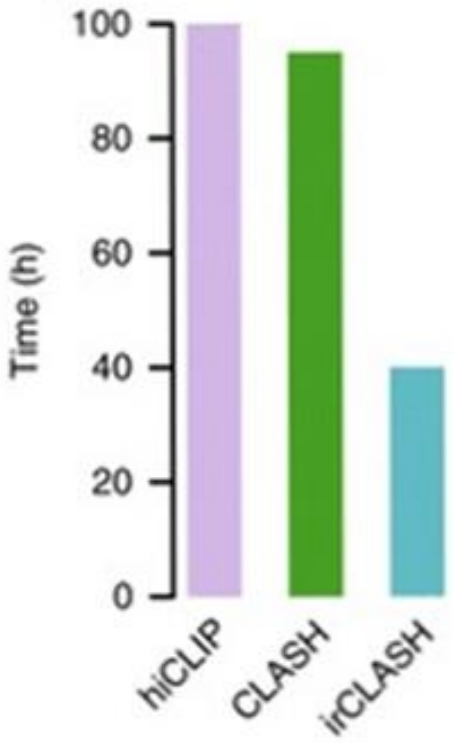


(Song et al., 2020)

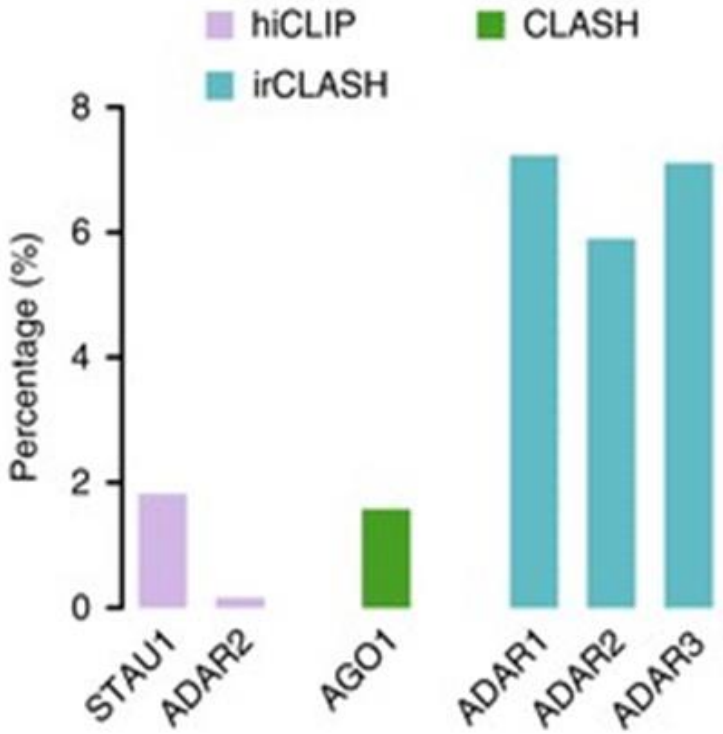
Why irCLASH?

- ~100h→ ~40h
- 7.2%, 5.9% and 7.1% of the uniquely mapped reads

Library construction time



Percentage of captured hybrid reads

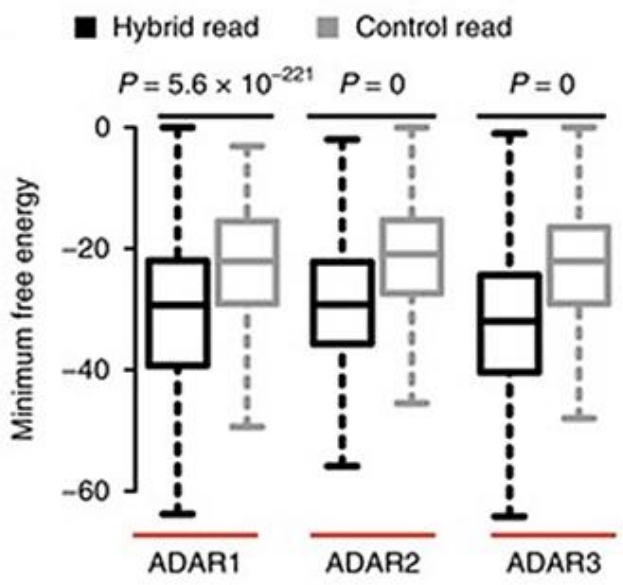


(Sugimoto, Y. et al., 2015)
(Helwak, A., 2013)
(Song et al., 2020)

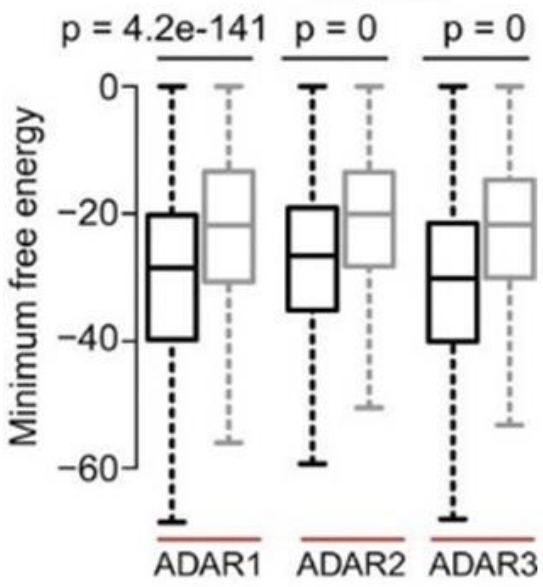
Validity of duplexes

Evaluation of thermodynamic stability

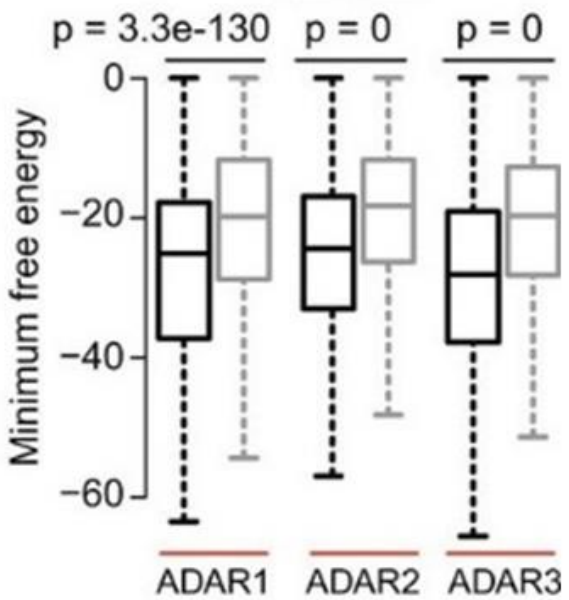
RNAhybrid



RNAstructure



RNAfold

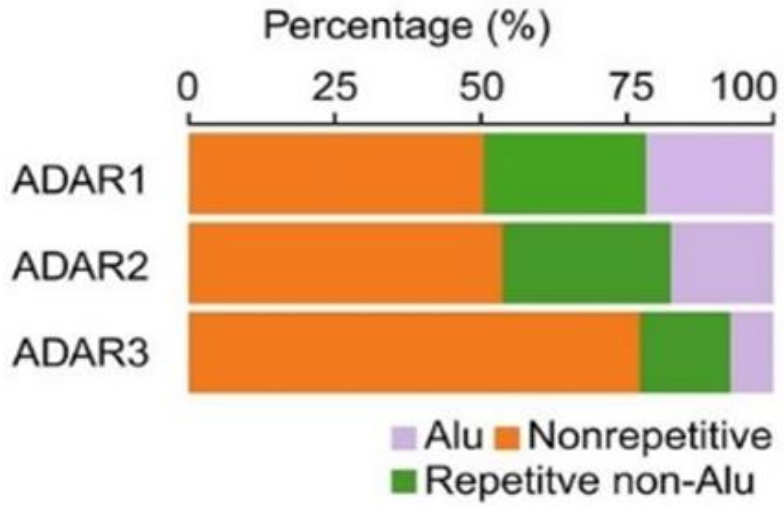


- MFE of ADARs hybrid reads lower than control read
- Hybrid reads have not been constructed randomly

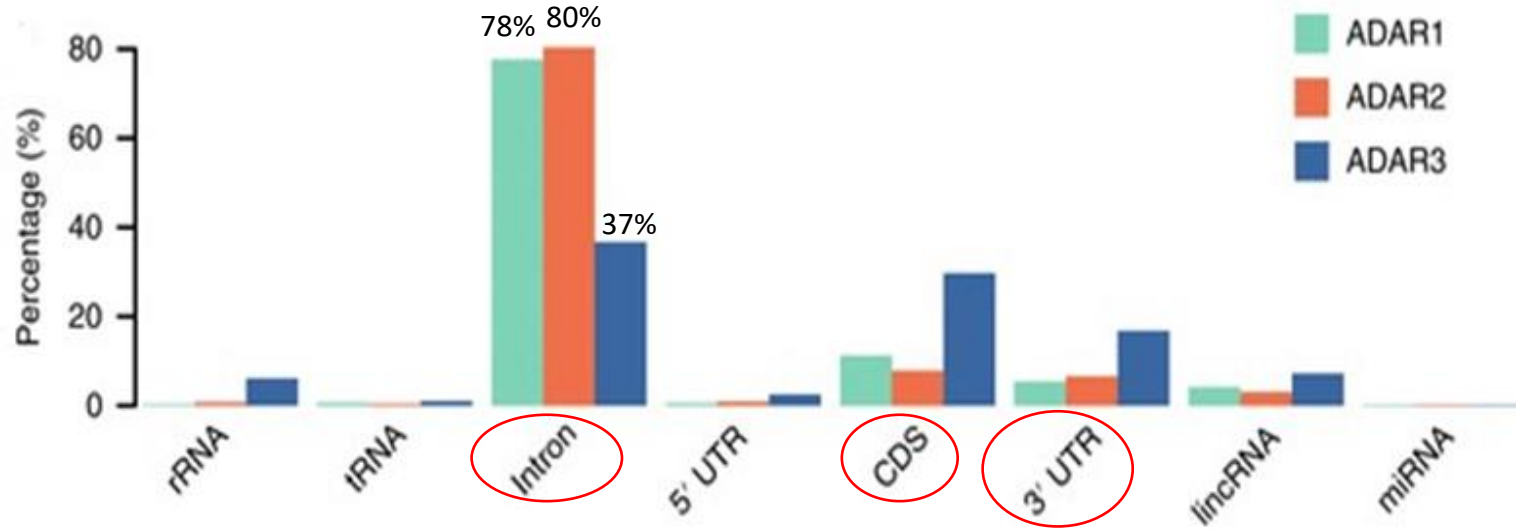
(Song et al., 2020)

Validity of duplexes

Content of ADARs hybrid reads



Genic location of ADARs hybrid reads



- Lower proportion of Alu repeats in ADAR3 hybrid reads than ADAR1 and ADAR2
- ADAR 1/2 hybrid reads → Intron
- ADAR 3 hybrid reads → Intron, CDS and 3' UTR
- ADAR3 bind to mature mRNA in the cytoplasm

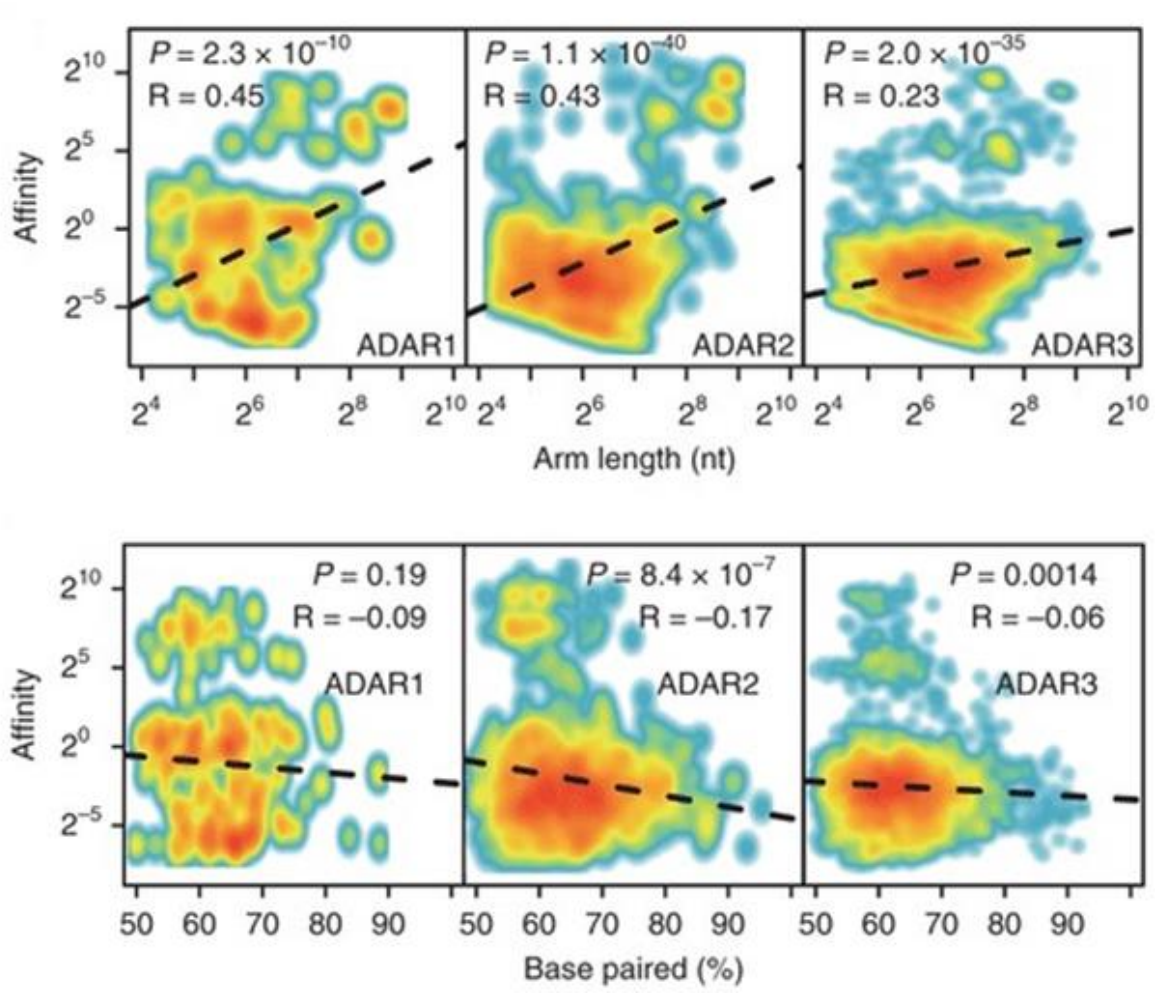
(Song et al., 2020)

ADAR binding affinity

- Affinity- Arm length
- Positive correlation

- Affinity- Base paired
- Weakly negative correlation
- ADAR2 bind non-perfectly matched dsRNA

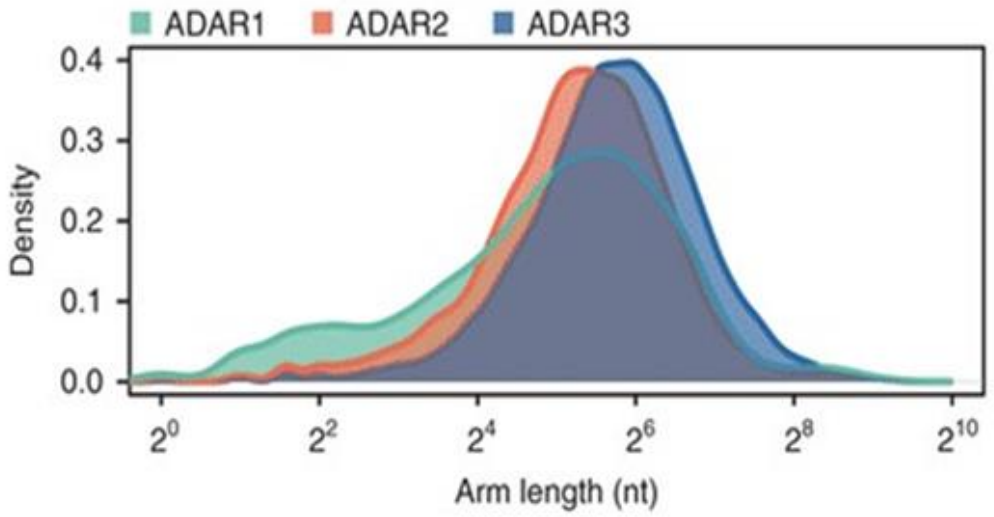
Relationships between affinity-arm length and affinity-base paired



(Song et al., 2020)

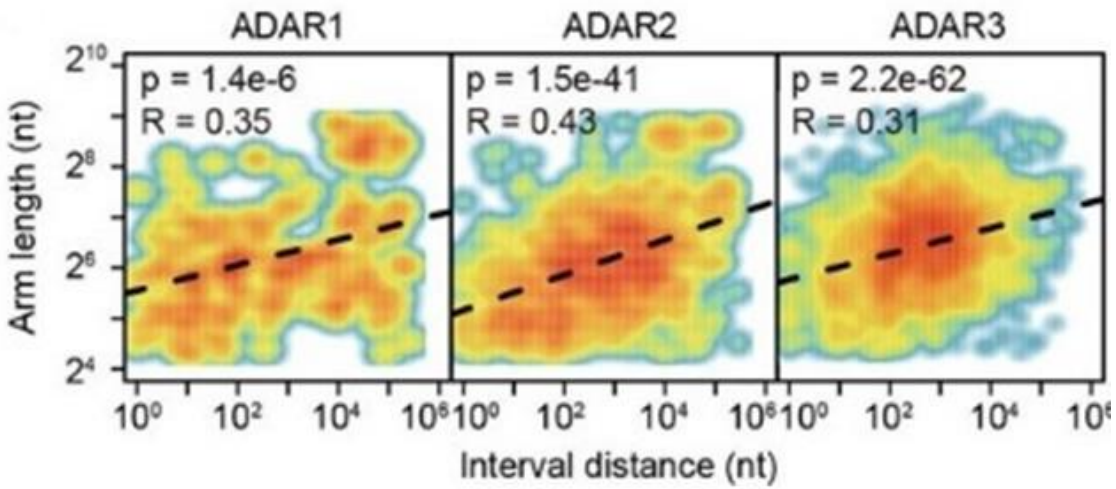
Arm length of ADAR substrates

Distribution of arm length of ADAR substrates



- Arm length ADAR substrates >>> 20bp

Relationship between arm length and interval distance

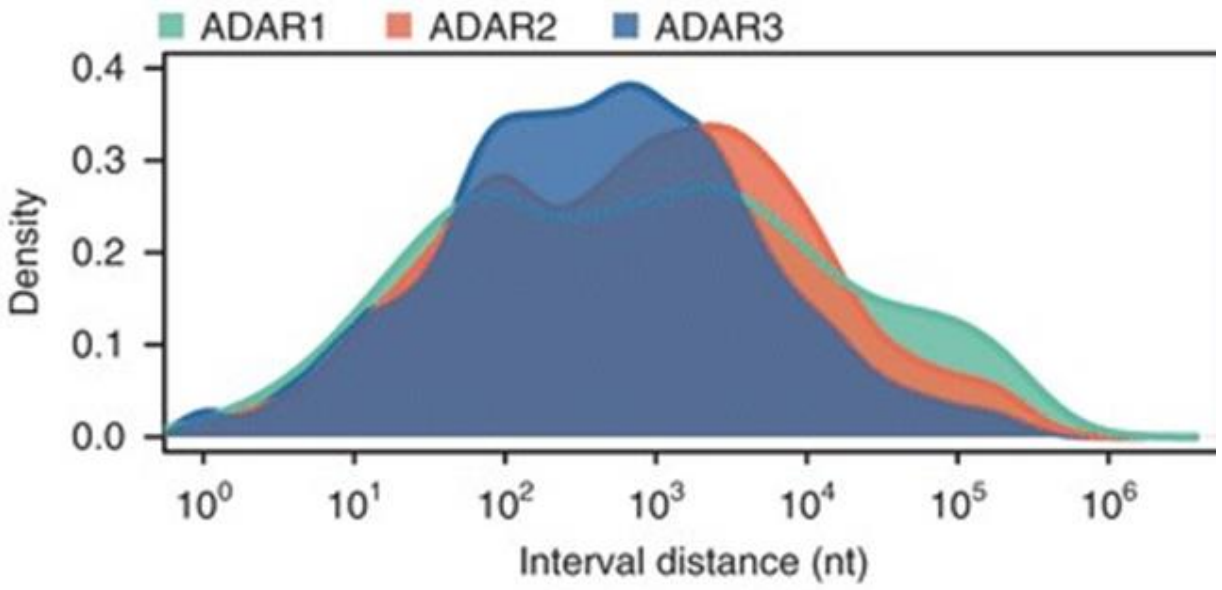


- Positive correlation
- Long-range interactions require longer arm length

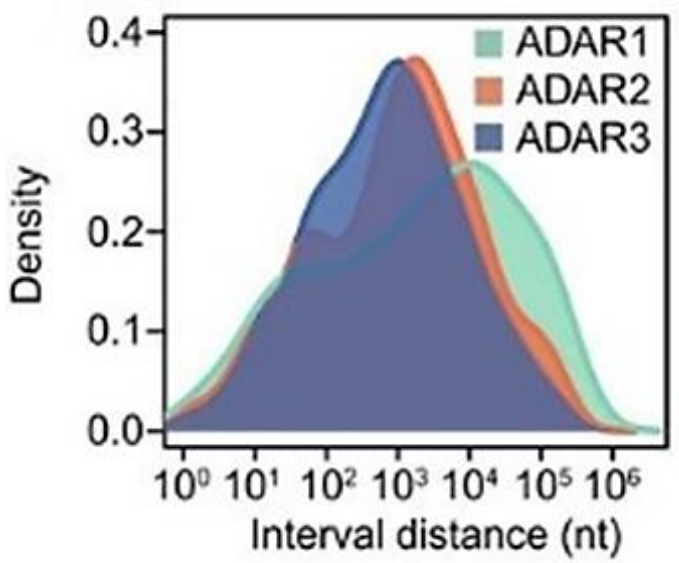
(Song et al., 2020)

Interval distances of ADAR substrates

Distribution of interval distance of non-Alu ADAR substrates



Distribution of interval distance of Alu ADAR substrates

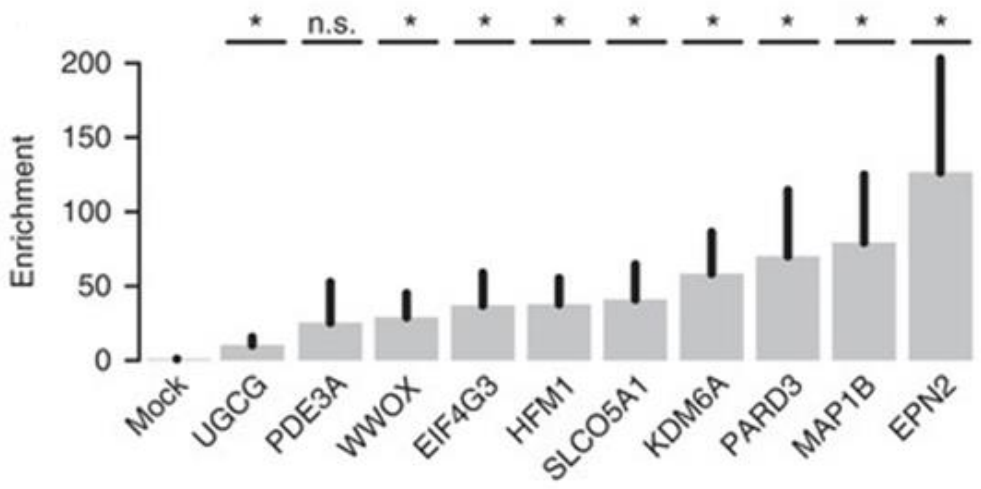


- ADAR3 has shorter interval distance
- Similar results were observed in Alu substrates

(Song et al., 2020)

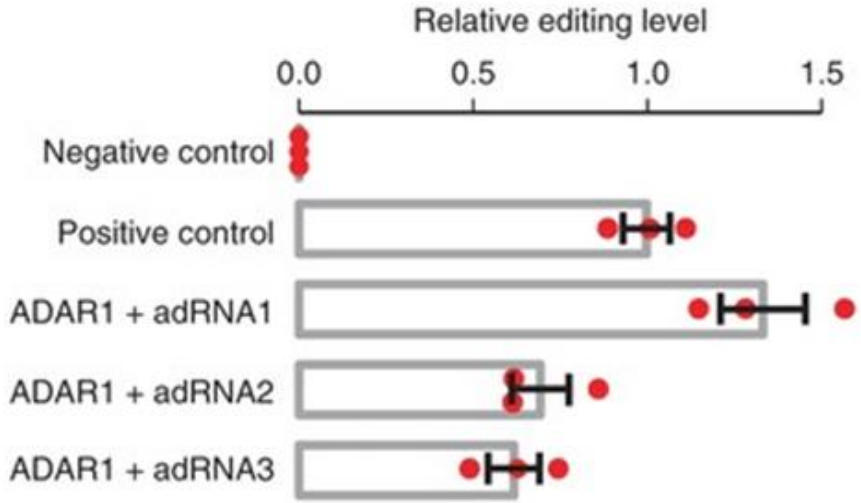
Recruiting of the ADAR1

Enrichment of RNA ADAR1 substrates in ADAR1 IP



- 10 random ADAR1 substrates
- 9 out of 10 substrates

RNA-editing efficiency of endogenous RAB7A

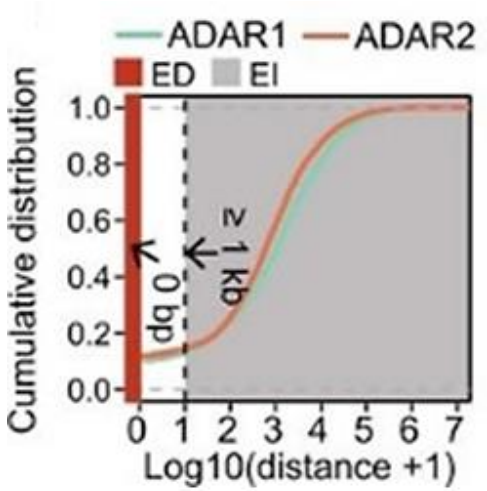


- 20-nt antisense sequence (adRNA)
- 3’ UTR RAB7A

(Song et al., 2020) (Katrekar , D. et al., 2019)

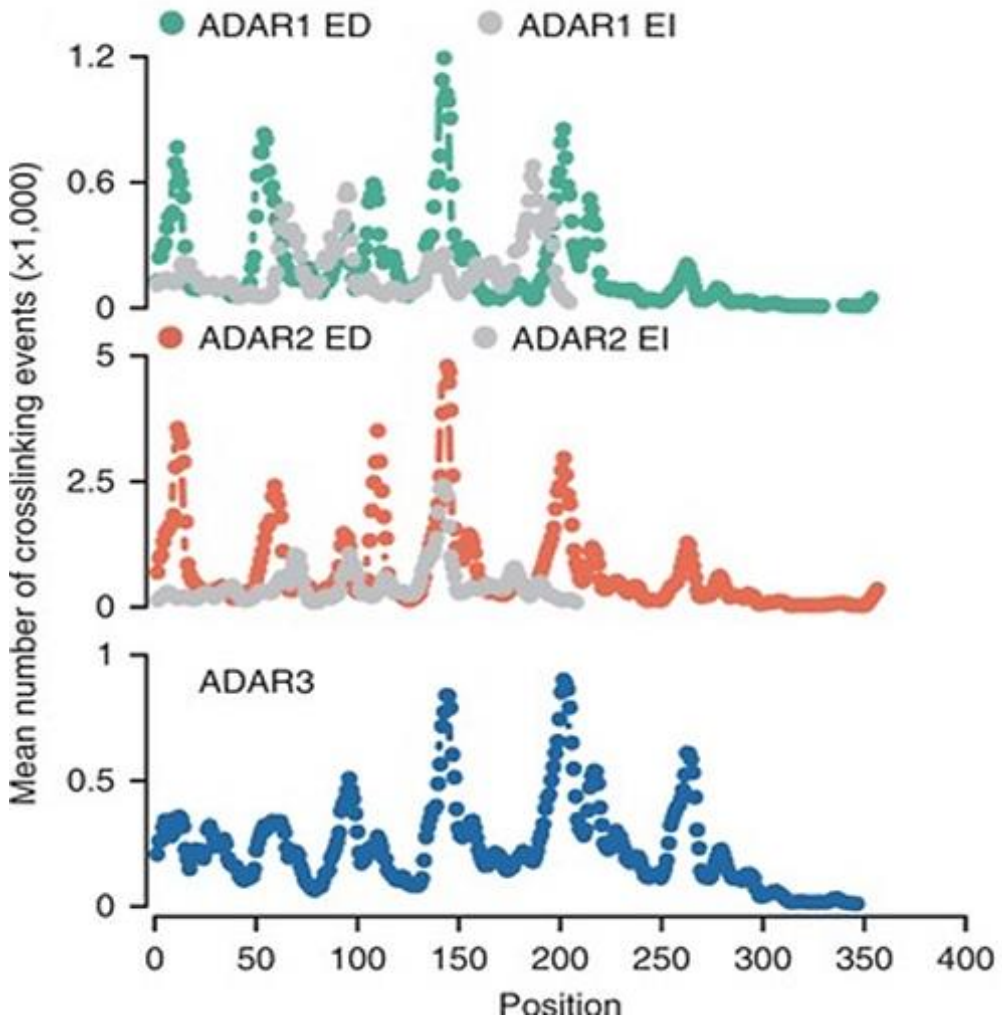
Crosslinking events along ADAR substrates

Separation of ADAR substrates



- Editing dependent (ED)
- Editing independent (EI)

Distribution of crosslinking events



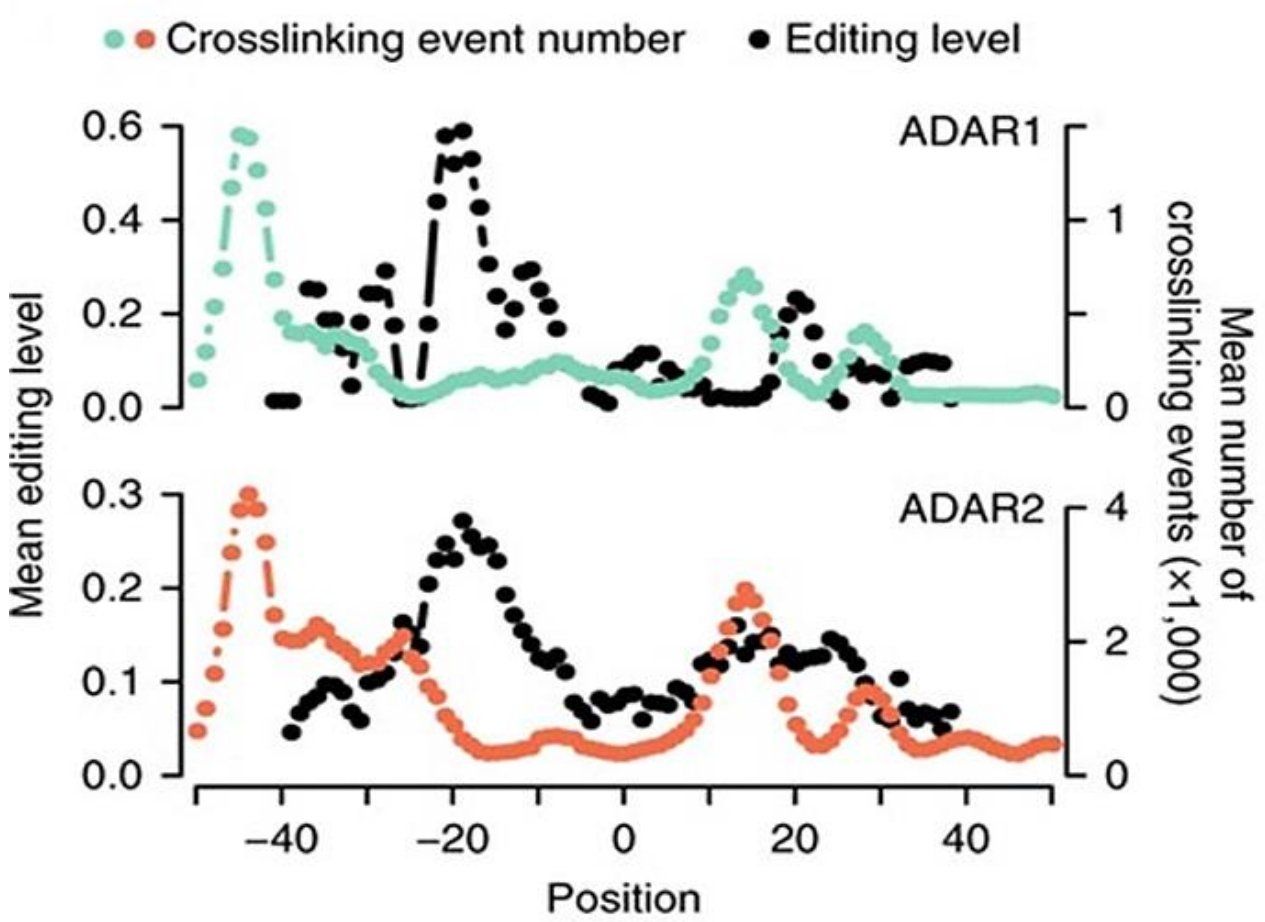
- Footprint with 50bp interval
- EI substrates with randomly separated peaks

(Song et al., 2020)

Crosslinking events along ADAR substrates

- Higher editing activity in 5’ region
- 10-30bp upstream
- Flanking amino acids of ADARs

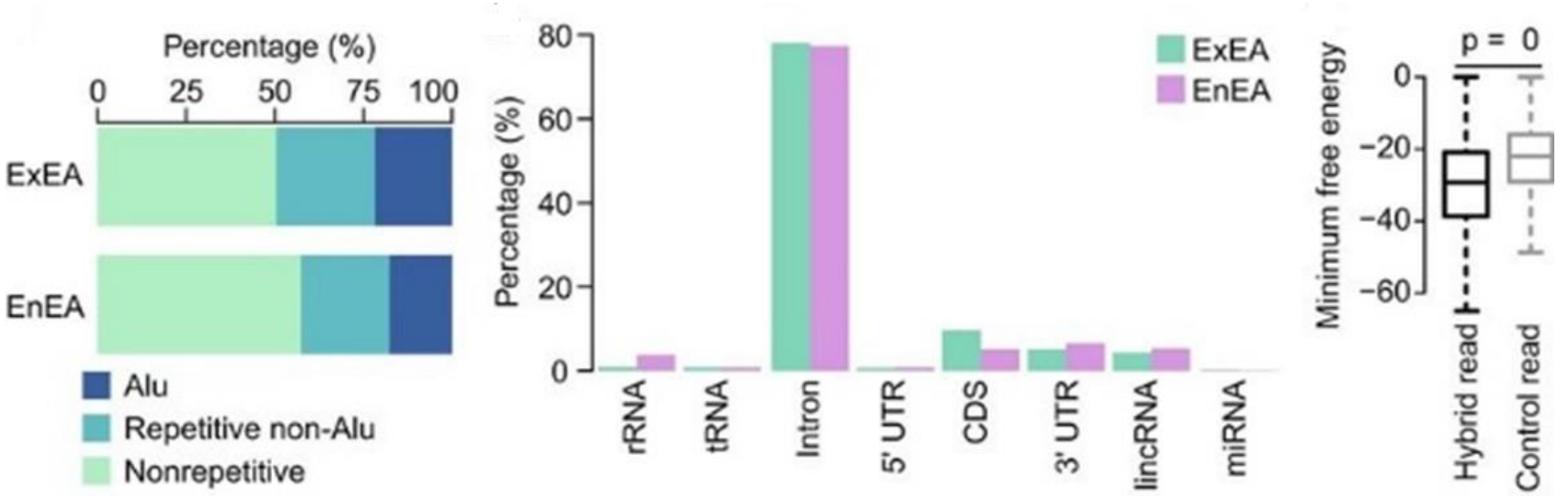
Editing activity and crosslinking events across editing-dependent substrates



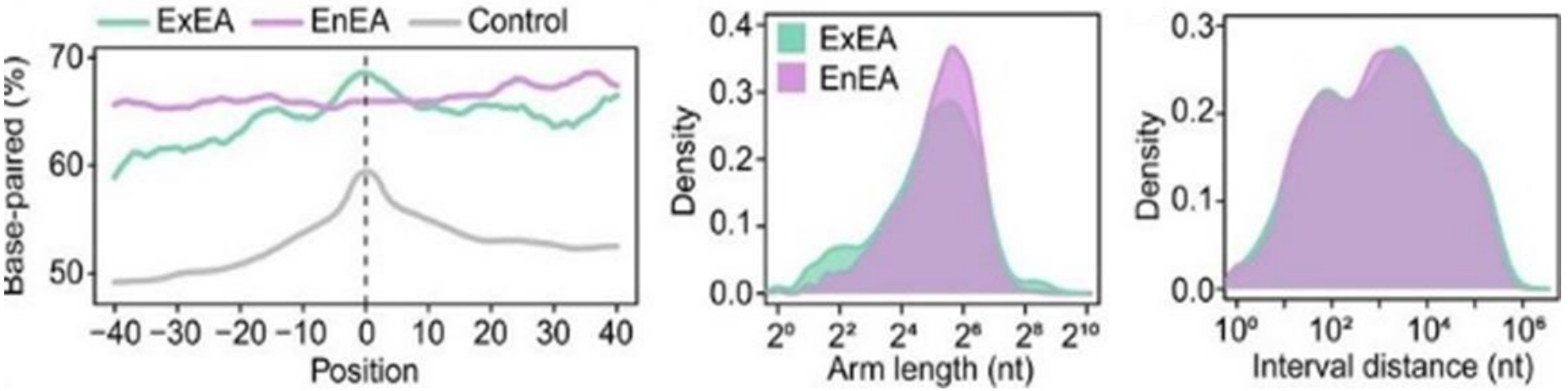
(Song et al., 2020)

Comparison of EnEA with ExEA

Comparison of RNA substrates bound by endogenously (EnEA) and exogenously expressed ADAR1 (ExEA)



- Same repeat features genic location
- MFE hybrids reads < MFE control reads

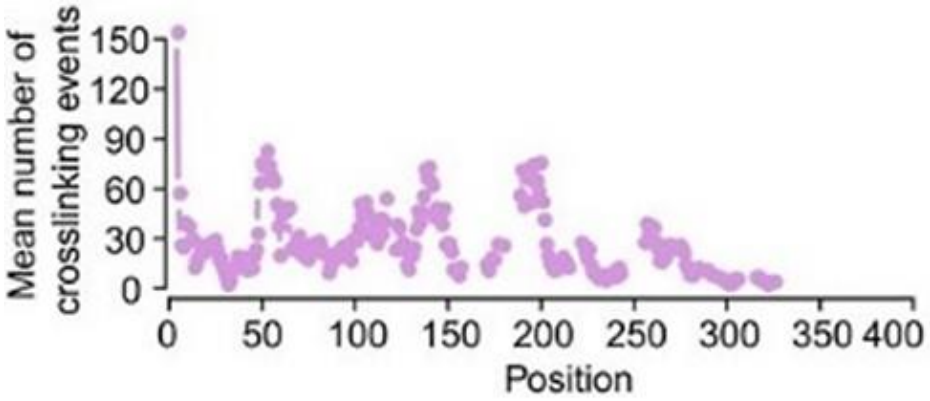


- Higher percentage of base-paired than the control
- Similar interval distances and arm length

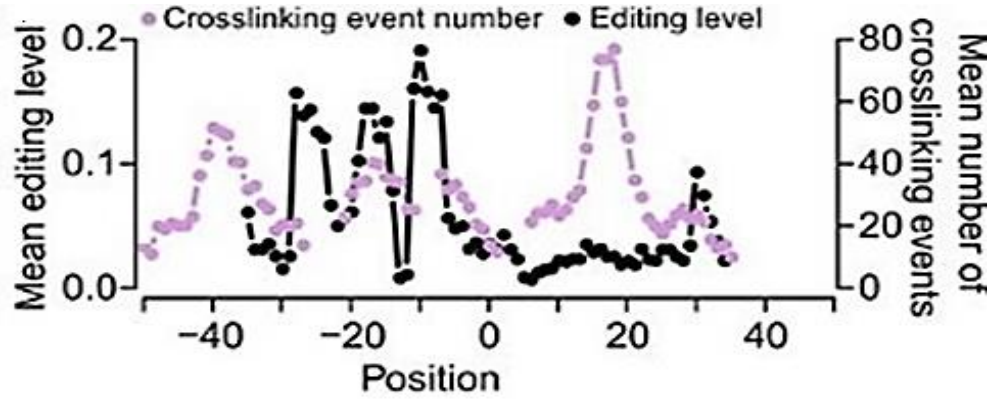
(Song et al., 2020)

Crosslinking events of EnEA substrates

Distribution of crosslinking events of EnEA substrates



Editing activity and crosslinking events EnEA substrates

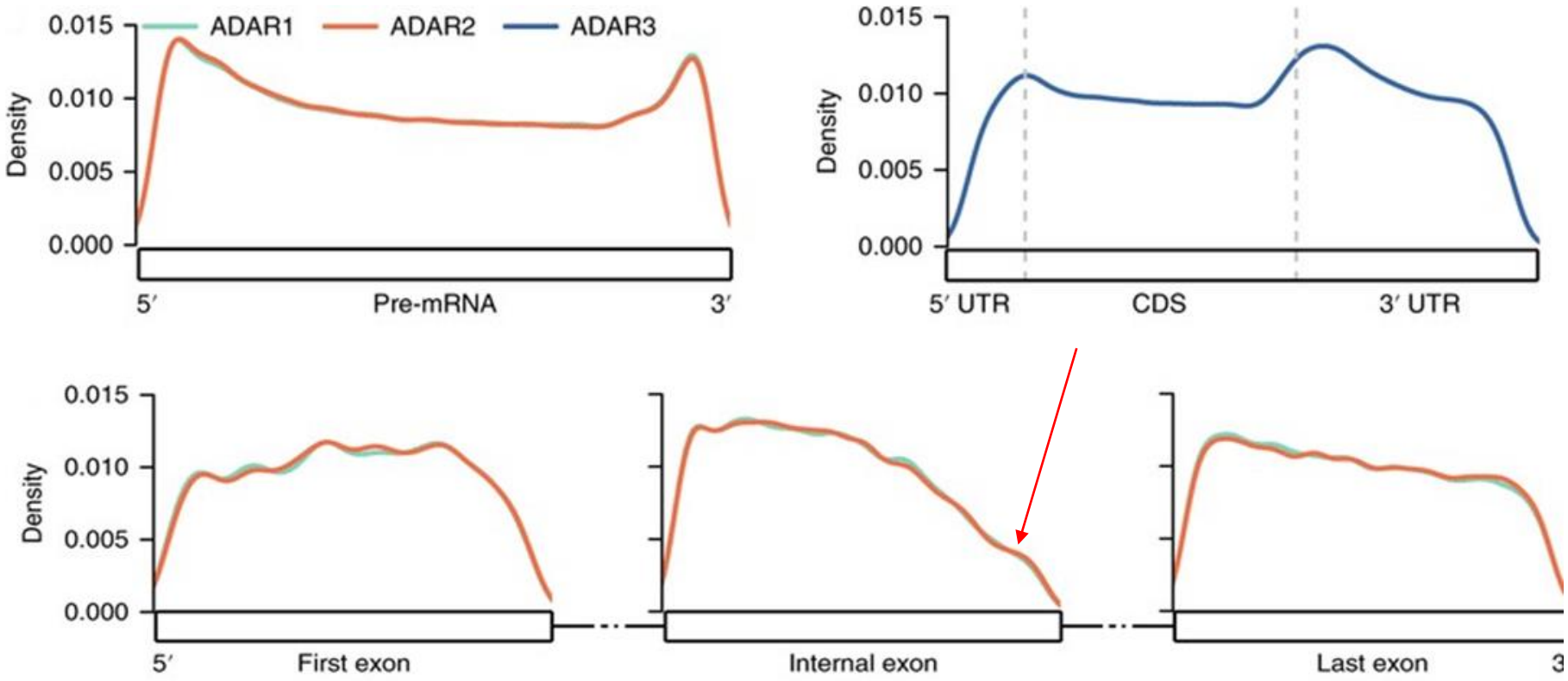


- Multiple consecutive peaks for editing-dependent EnEA substrates with a 50-bp interval
- More crosslinking events 10-30 bp upstream the regions with high editing activity

(Song et al., 2020)

The binding profiles of ADARs on pre-mRNA or mRNA

The distribution of ADARs binding sites

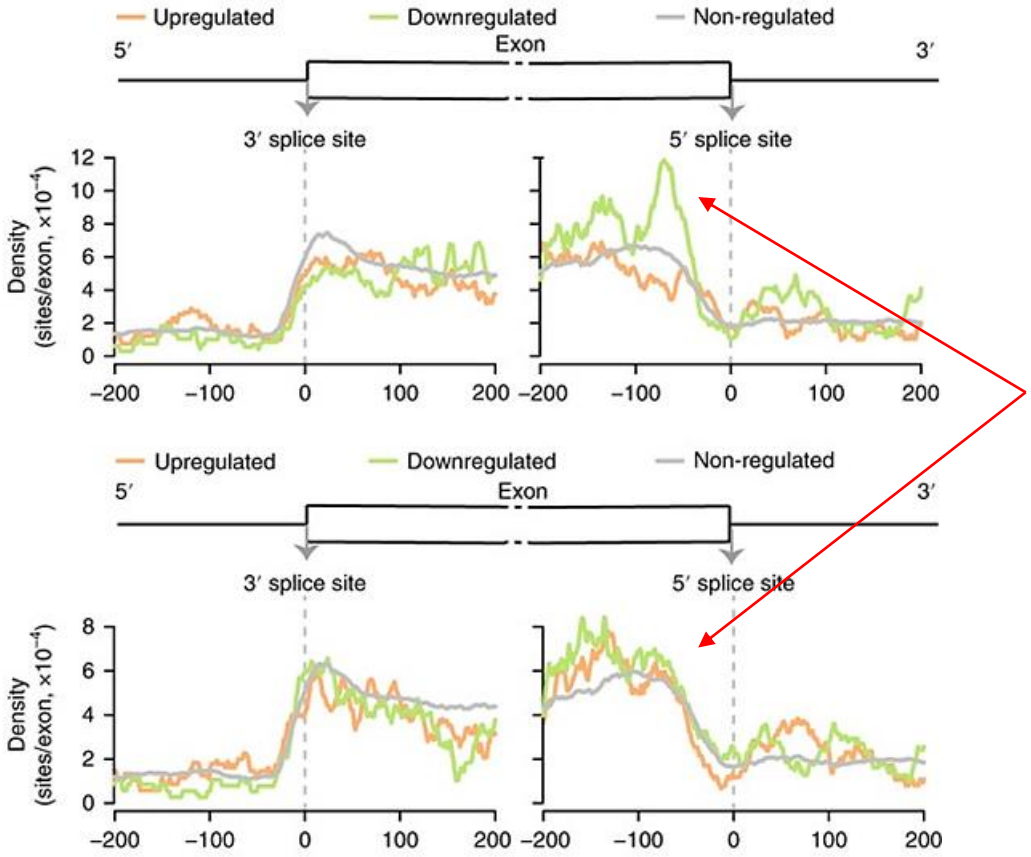


- ADAR1 and ADAR2 bind more to 3’UTR and 5’UTR
- ADAR3 prefer to bind to 3’UTR
- ADARs don’t prefer to bind the 3’ end of the internal exons

(Song et al., 2020)

Effect of ADARs in splicing

The density of ADAR1 and ADAR2 binding sites near 5′ and 3′ splice sites of exons



(Song et al., 2020)

RNA editing in nascent RNA affects pre-mRNA splicing

Yun-Hua Esther Hsiao [1], Jae Hoon Bahn [2], Yun Yang [2], Xianzhi Lin [2], Stephen Tran [3], Ei-Wen Yang [2], Giovanni Quinones-Valdez [1], Xinshu Xiao [1 2 3 4]

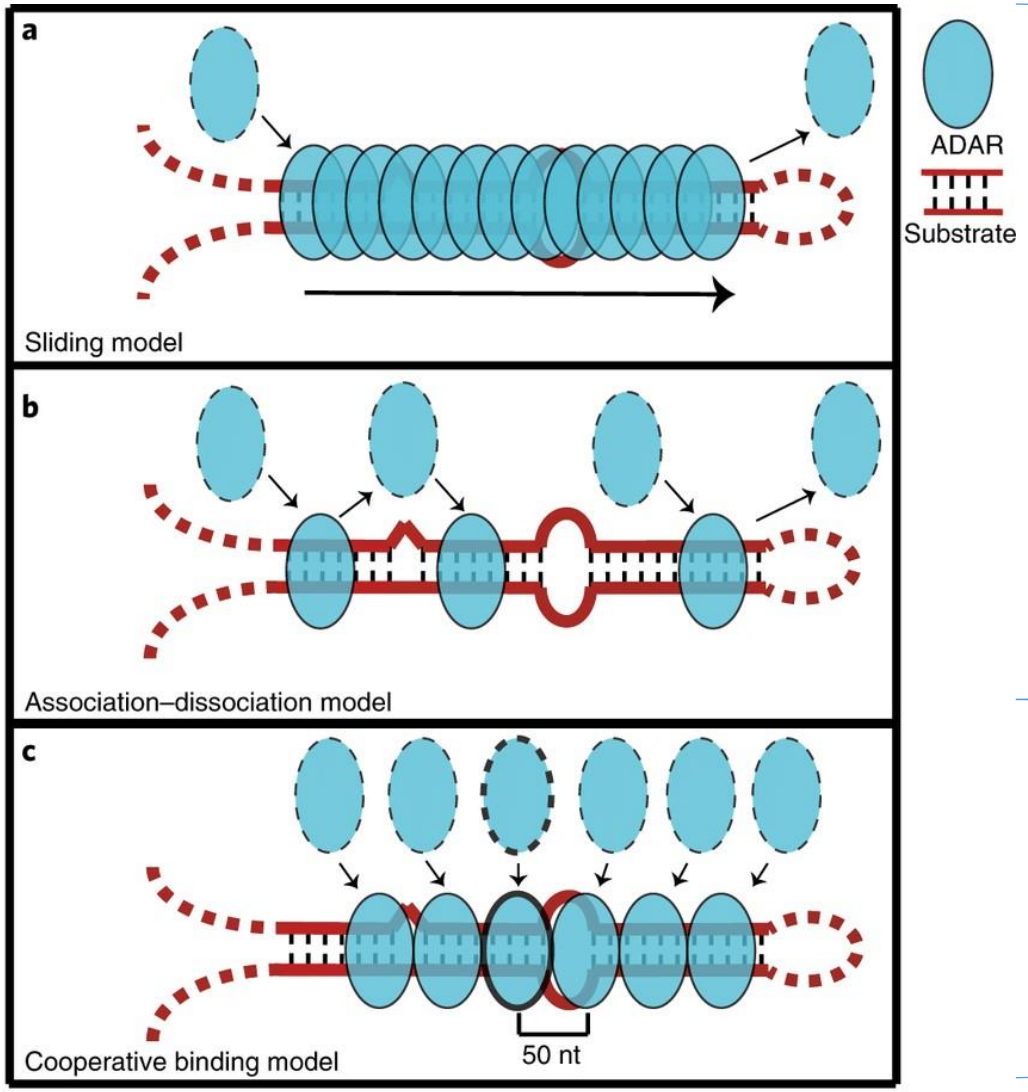
Affiliations + expand

PMID: 29724793 PMCID: PMC5991522 DOI: 10.1101/gr.231209.117

- Upstream of 5′ splice sites of the exons
- ADAR1 and ADAR2 repress splicing

ADARs interactions with long dsRNAs in vivo

Suggesting models of ADARs-mediated binding and editing



- the crosslinking events will be relatively evenly distributed across ADAR substrates
- no peaks will be observed

- crosslinking events unevenly distributed across ADAR substrates
- consecutive peaks may be observed
- more likely to be the way that ADAR proteins bind dsRNA substrates

(Song et al., 2020)

Main points

- irCLASH maps RNA substrates recognized by human ADARs and has shown higher efficiency than previous methods
- ADAR binding affinities of less base-paired dsRNAs are not only comparable but even higher than that of more based-paired dsRNAs
- ADAR proteins bind dsRNA substrates tandemly in vivo, each with a 50-bp footprint
- The 5’ region of a substrate tend to have a high editing activity
- ADAR1 and ADAR2 may repress splicing when binding to the region upstream of 5’ splice sites
- This transcriptome-wide atlas of ADAR substrates and the features governing RNA editing observed in this study will assist in the rational design of guide RNAs for ADAR-mediated RNA base editing

References

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