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How the epitranscriptome is changing our world

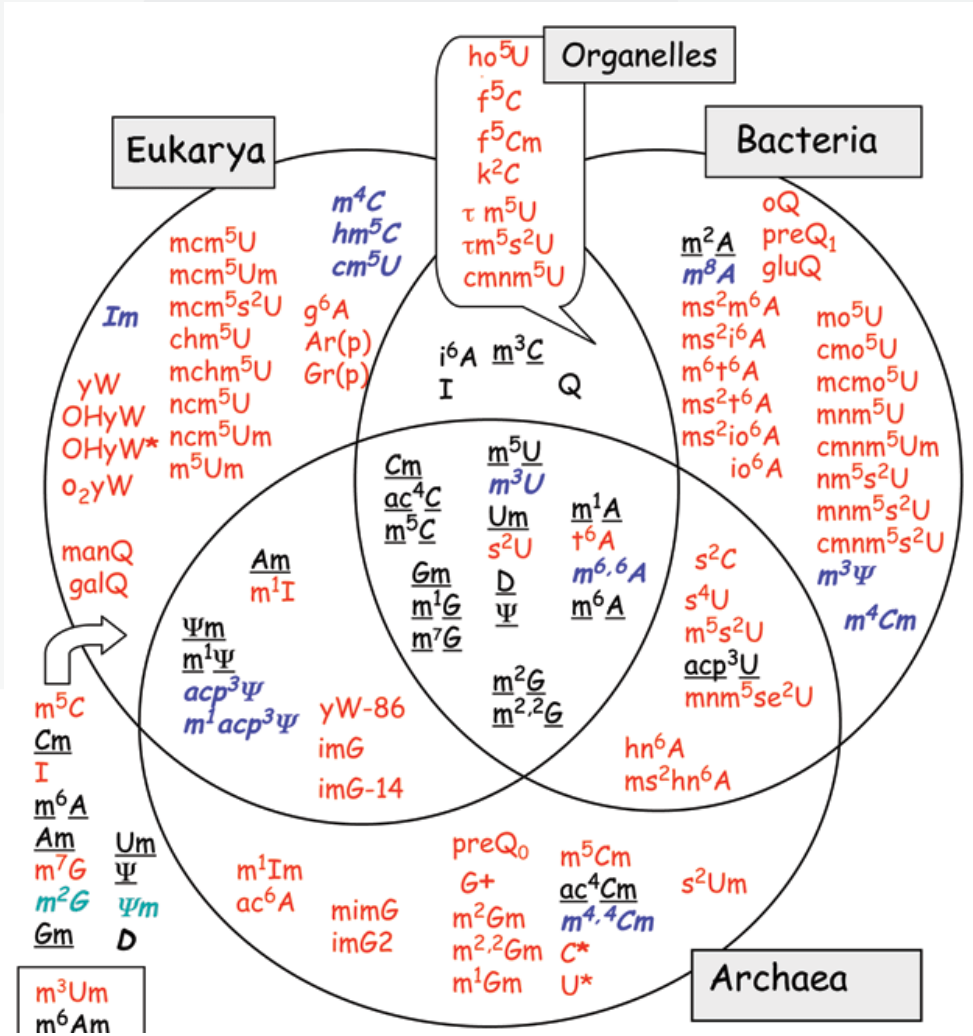
Mary O'Connell

What is the epitranscriptome?

Guess!

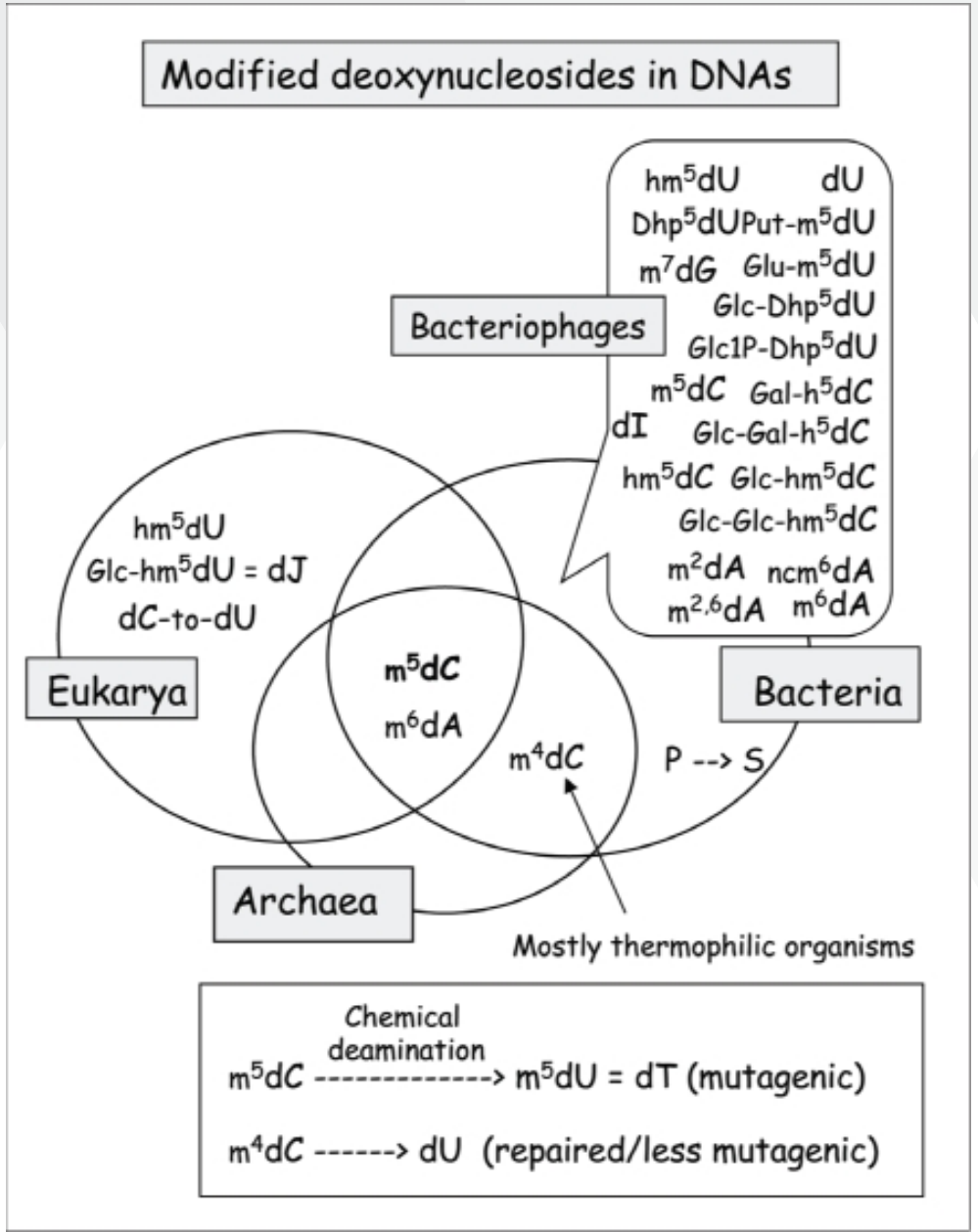
RNA modification

>170 RNA modifications



Henri Grosjean

Modifications in DNA



WHY?

Henri Grosjean

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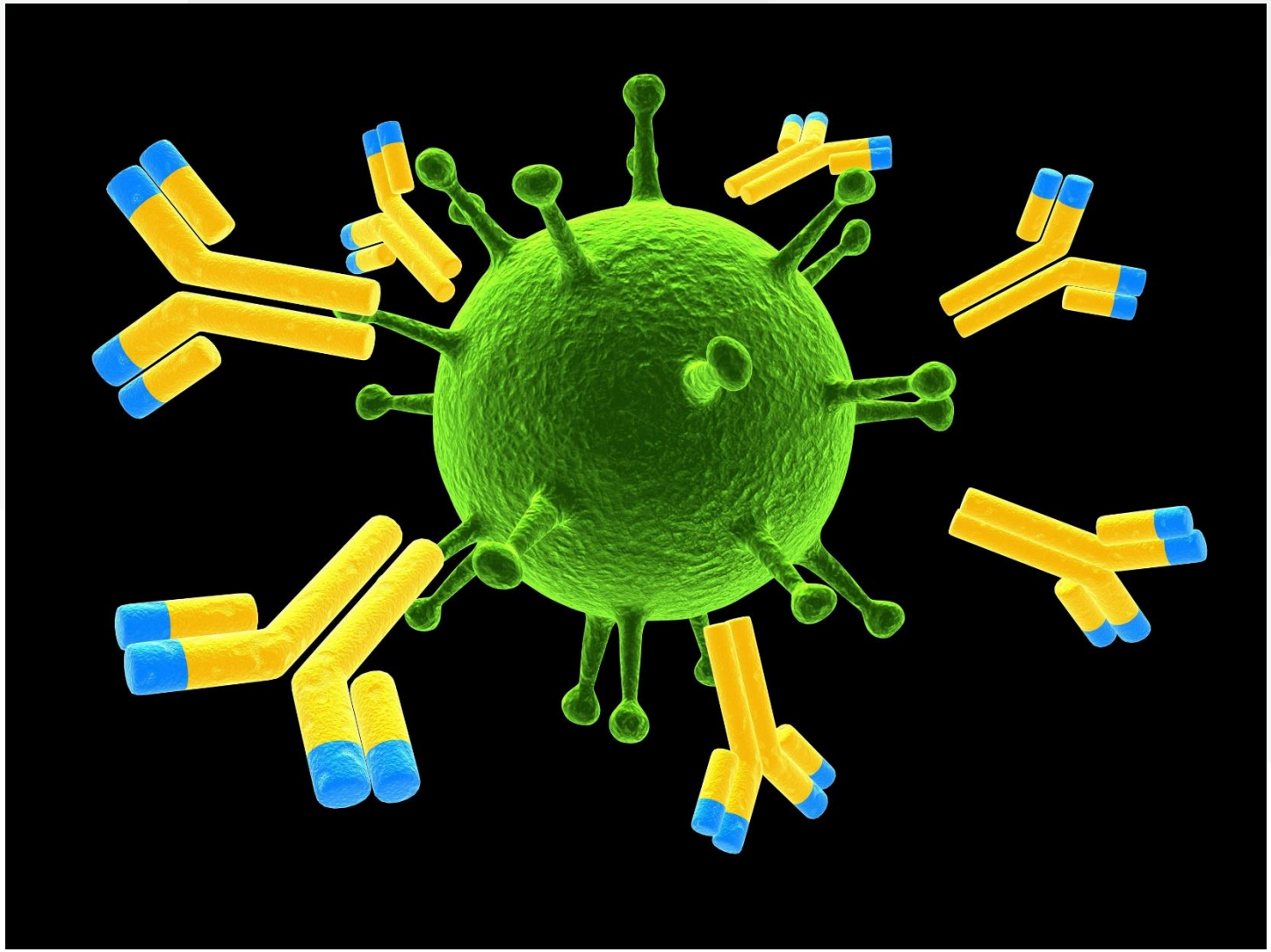
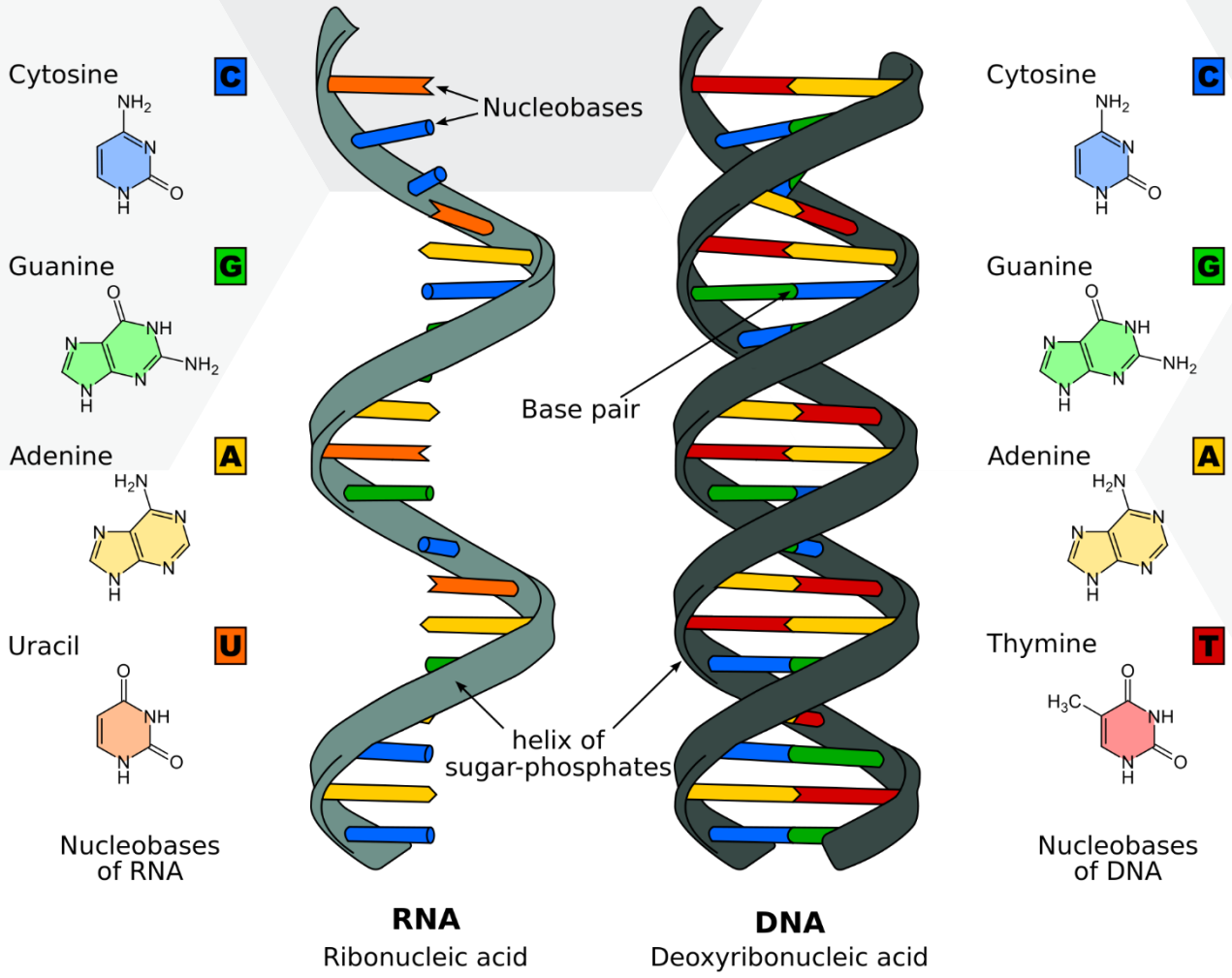
Antiboby/Antigen interaction



Problem with nucleic acids



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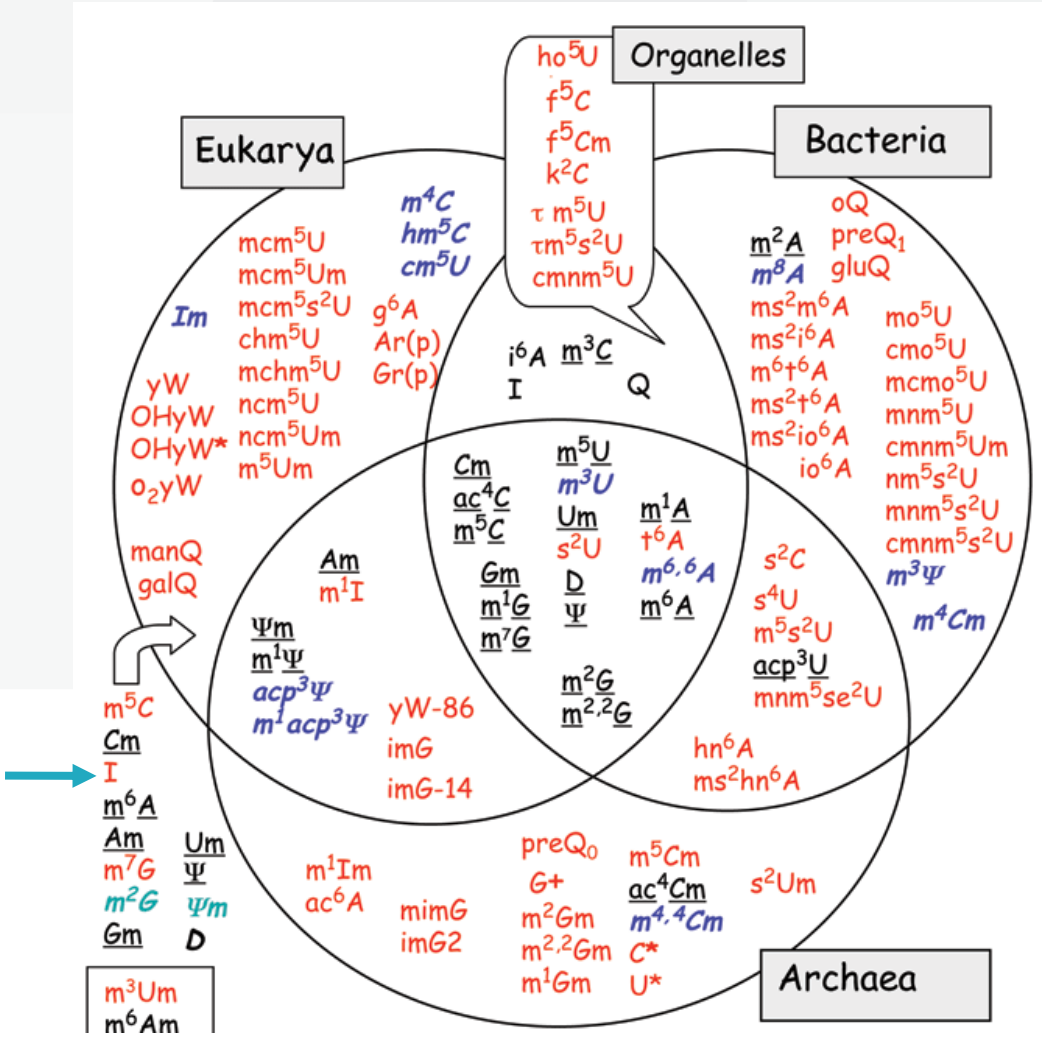
Approximately 150 RNA modifications



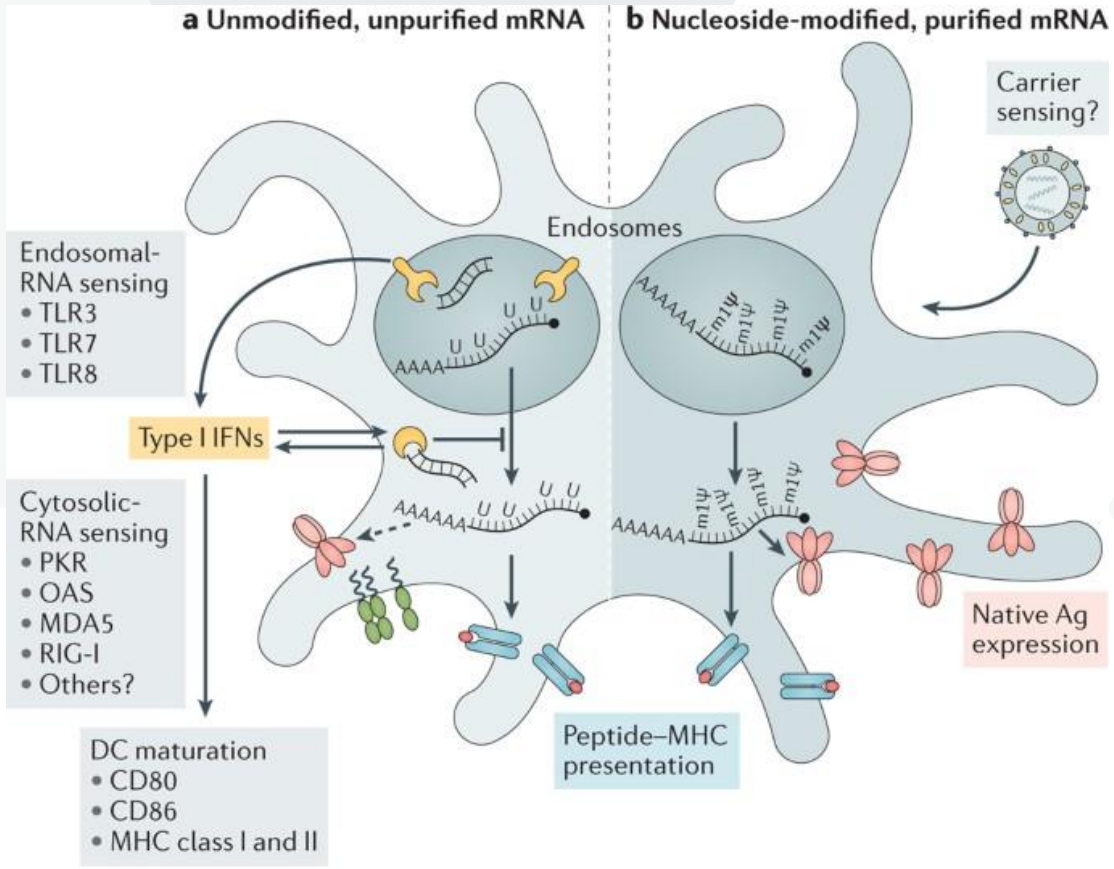
Henri Grosjean

RNA vaccines

Katalin Kariko

Drew Weissman

Modified versus unmodified RNA



Nature Reviews | Drug Discovery

Fast vaccines

NEXT-GENERATION mRNA VACCINES

Computer-designed, a feature that makes them adaptable and rapidly scalable into millions of doses.

Rely on **genetic material called mRNA** to create an immune response against a specific antigen, or toxin—in this case, the coronavirus.

Production for the general population **can take just weeks** rather than the months required for conventional vaccines.

mRNA Vaccine

Components

mRNA (blueprint of protein)

Production

Faster because mRNA molecules are easier to produce

Process

Components are injected into the arm and serve as instructions for the body to make microbial protein

Traditional Vaccine

Components

Microbial protein or inactive microbe

Production

Slower and more difficult to produce the right type of protein

Process

Components are made in a lab and injected into the arm to stimulate immune response

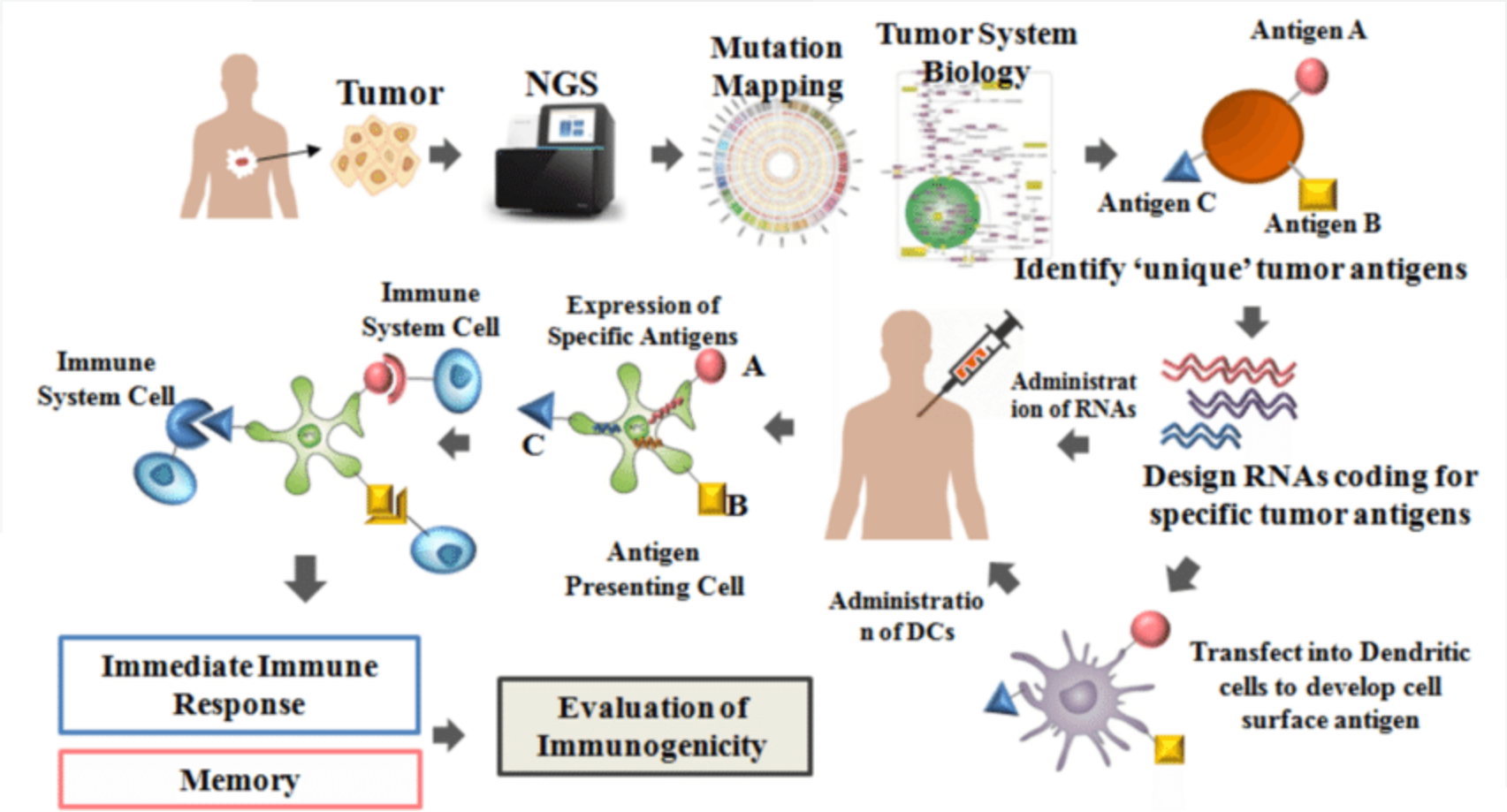
R & D

Antigen determined for immune stimulation

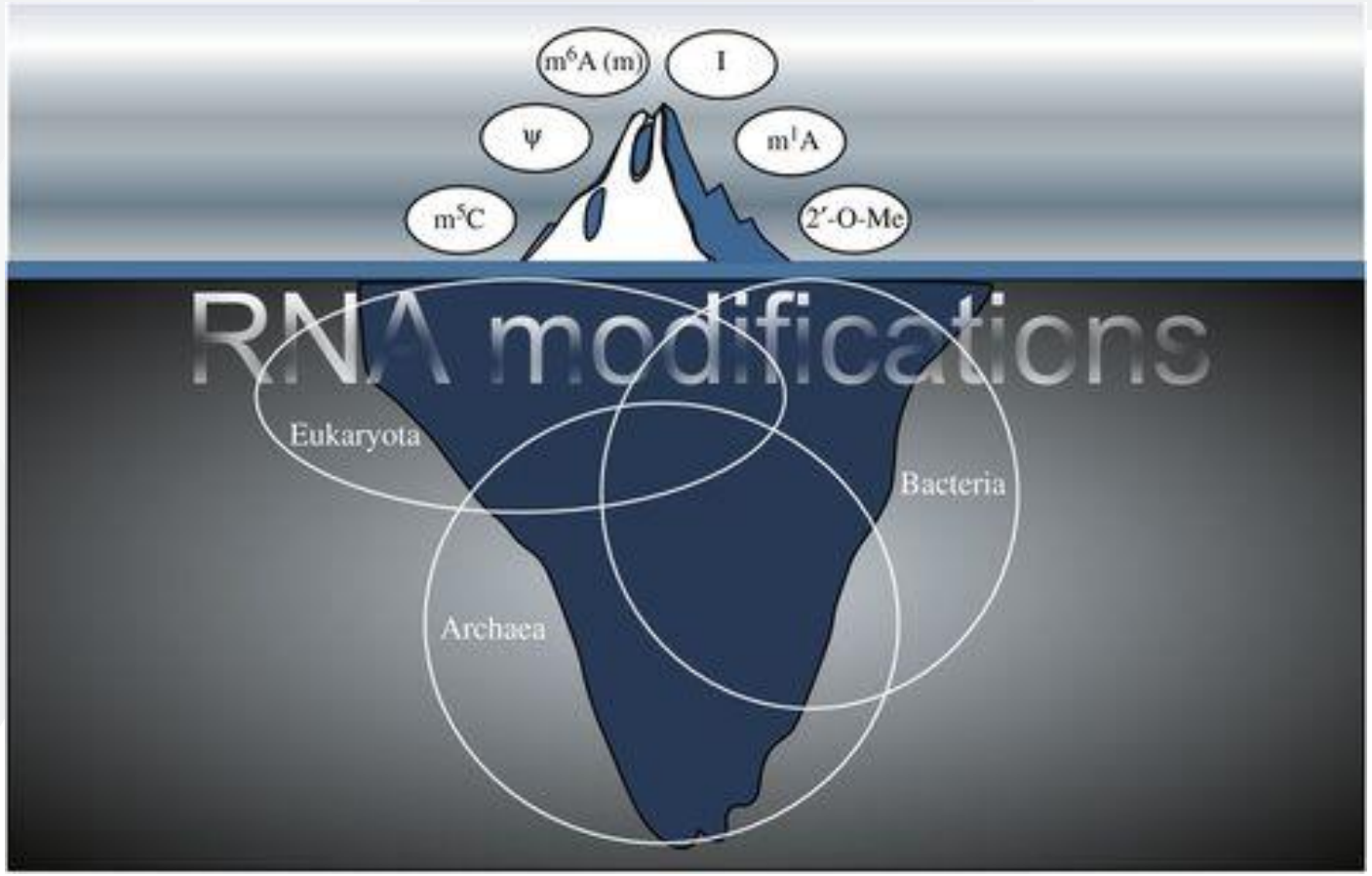
Result

Teaches the body to protect itself against a microbe

Development of personalized RNA-based cancer vaccines

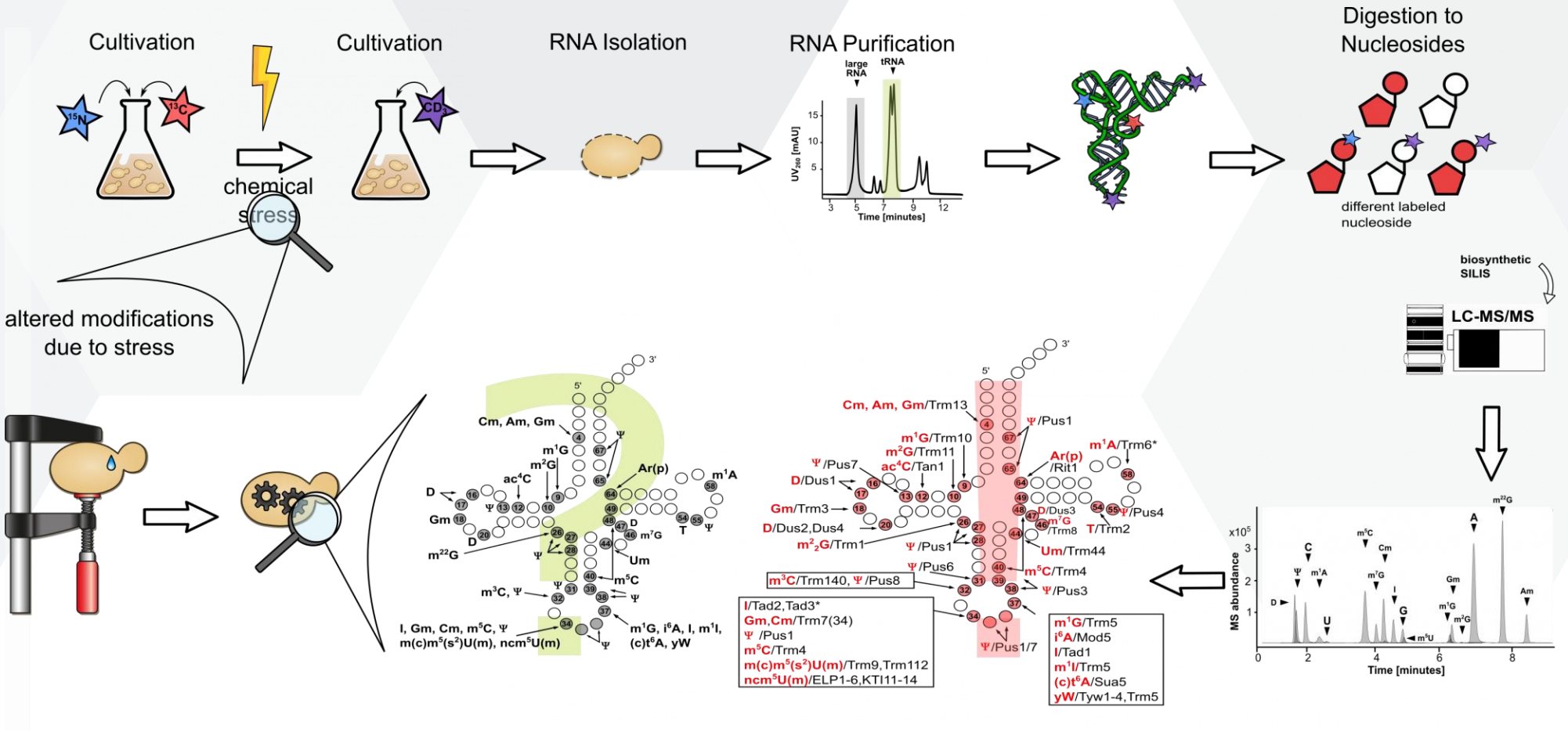


Challenges

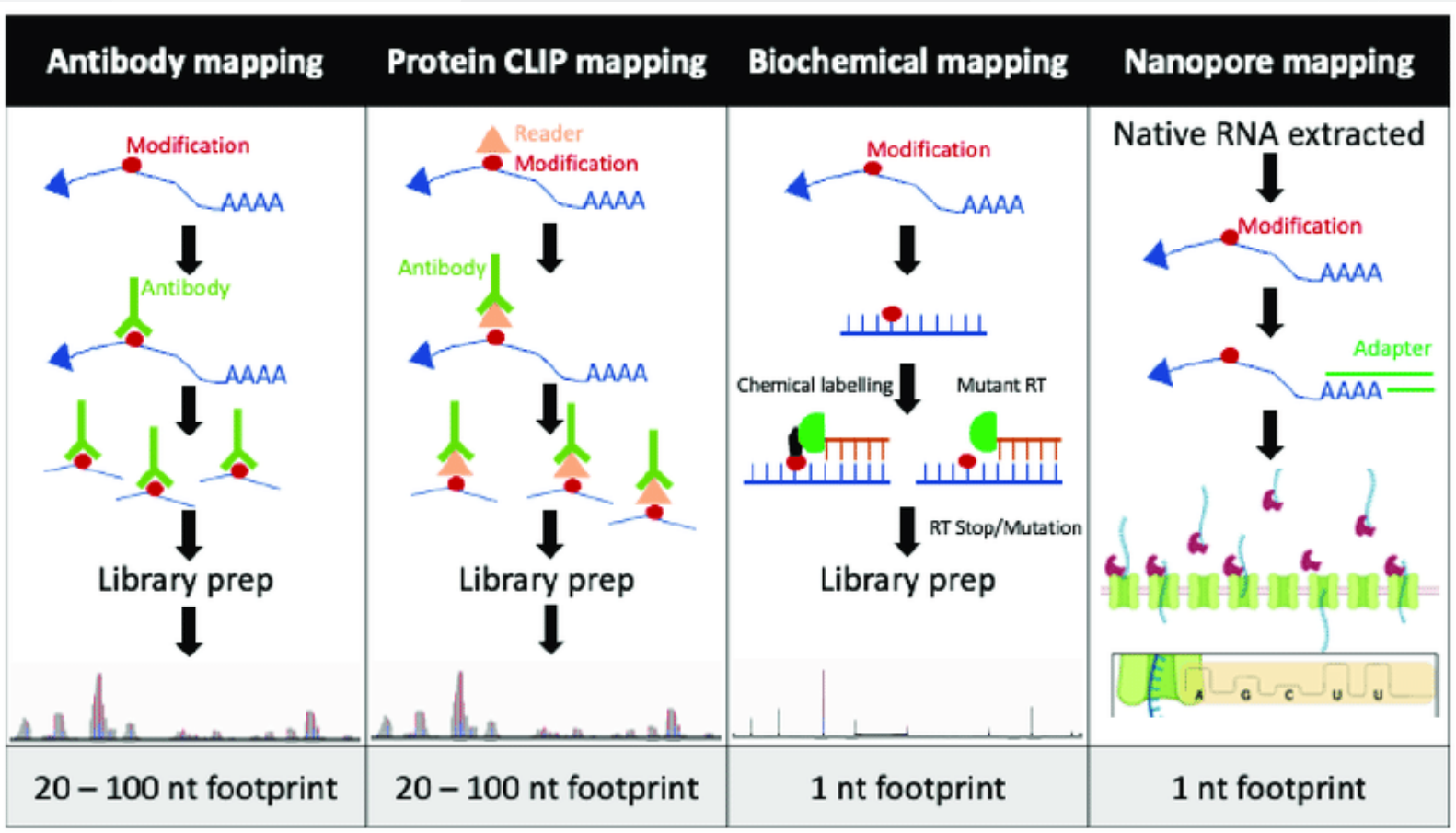


Problems with detection

Amount of starting material



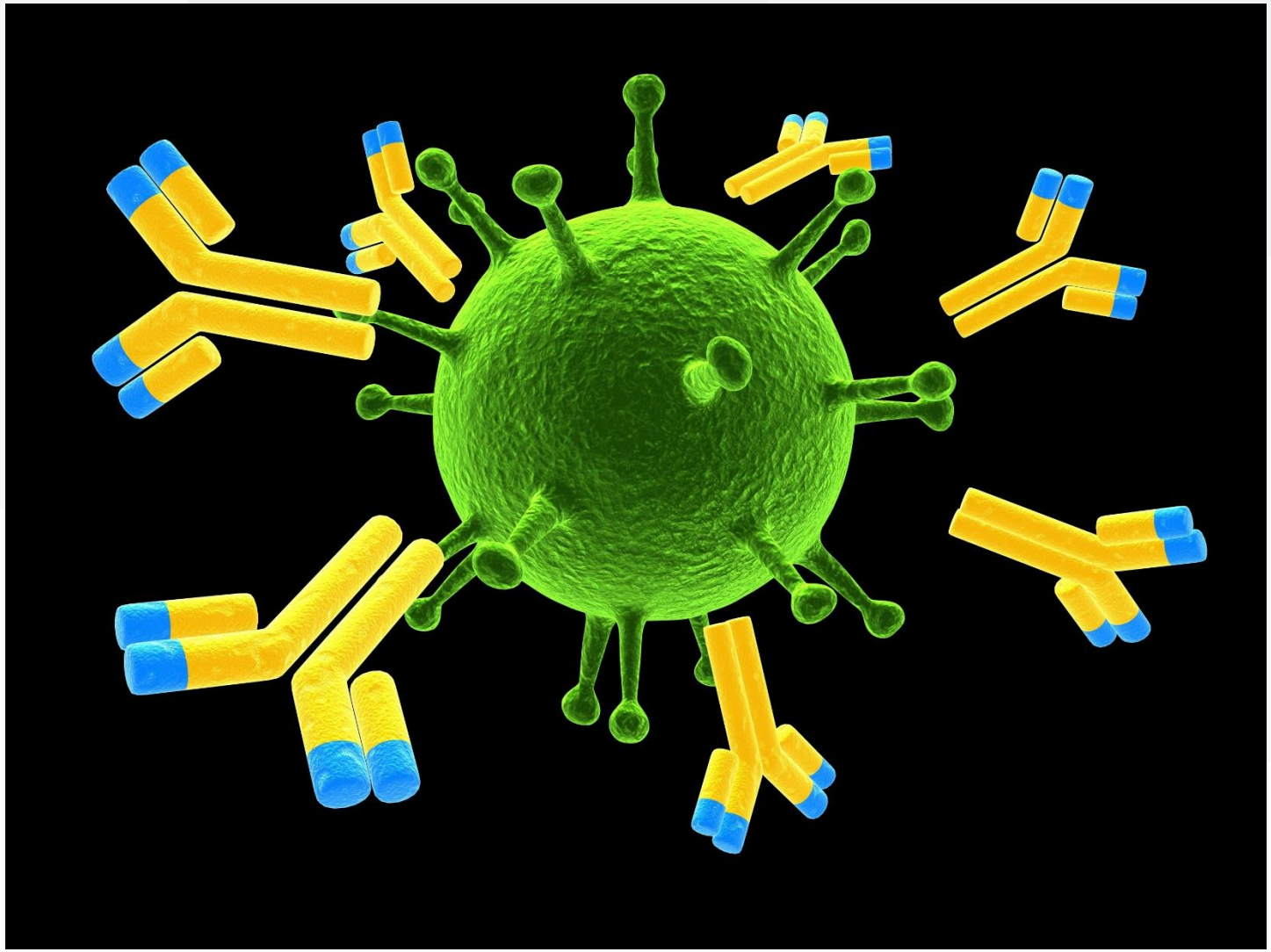
Current techniques



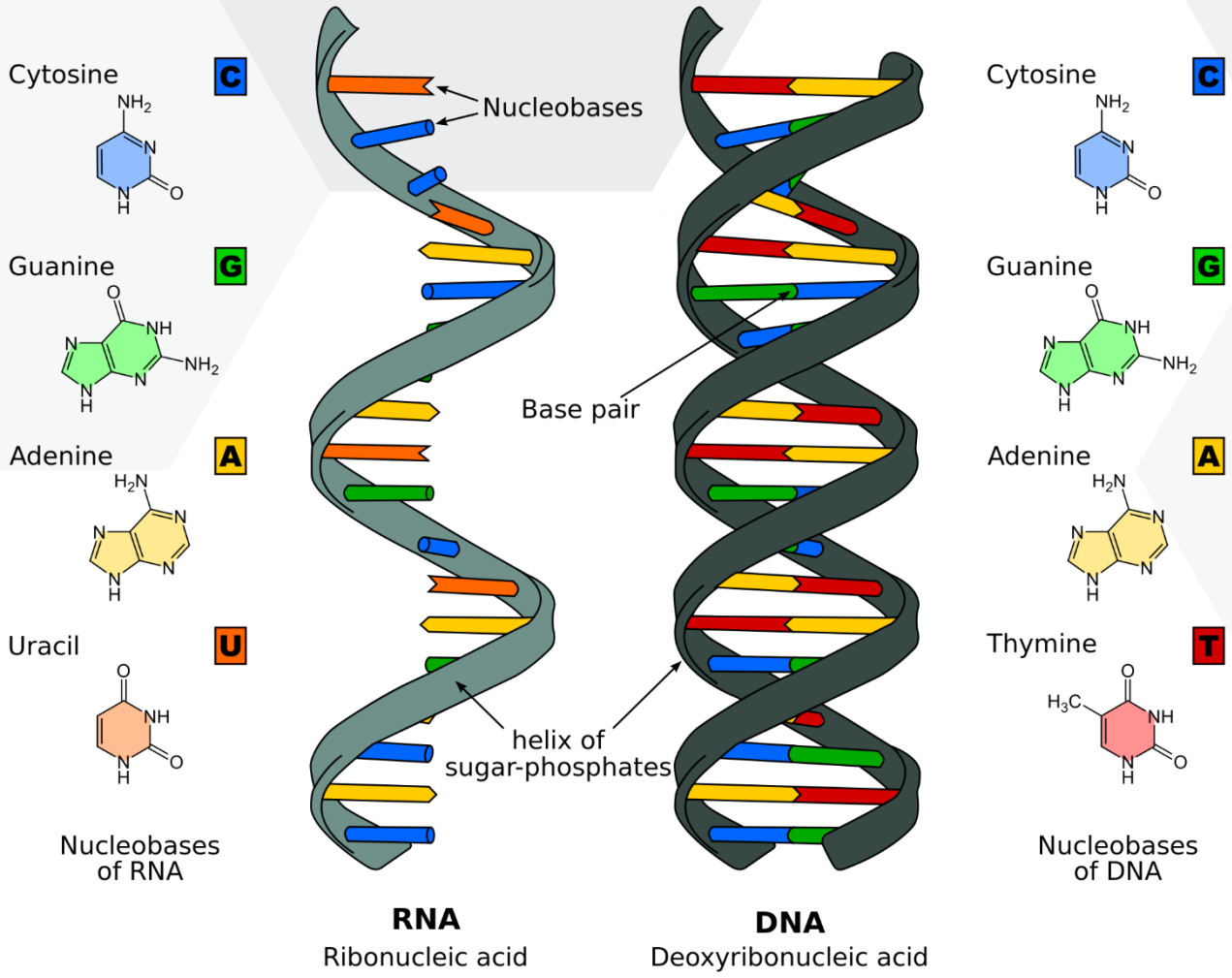
Problem with antibody specificity

What we do

Antiboby/Antigen interaction



Problem with nucleic acids



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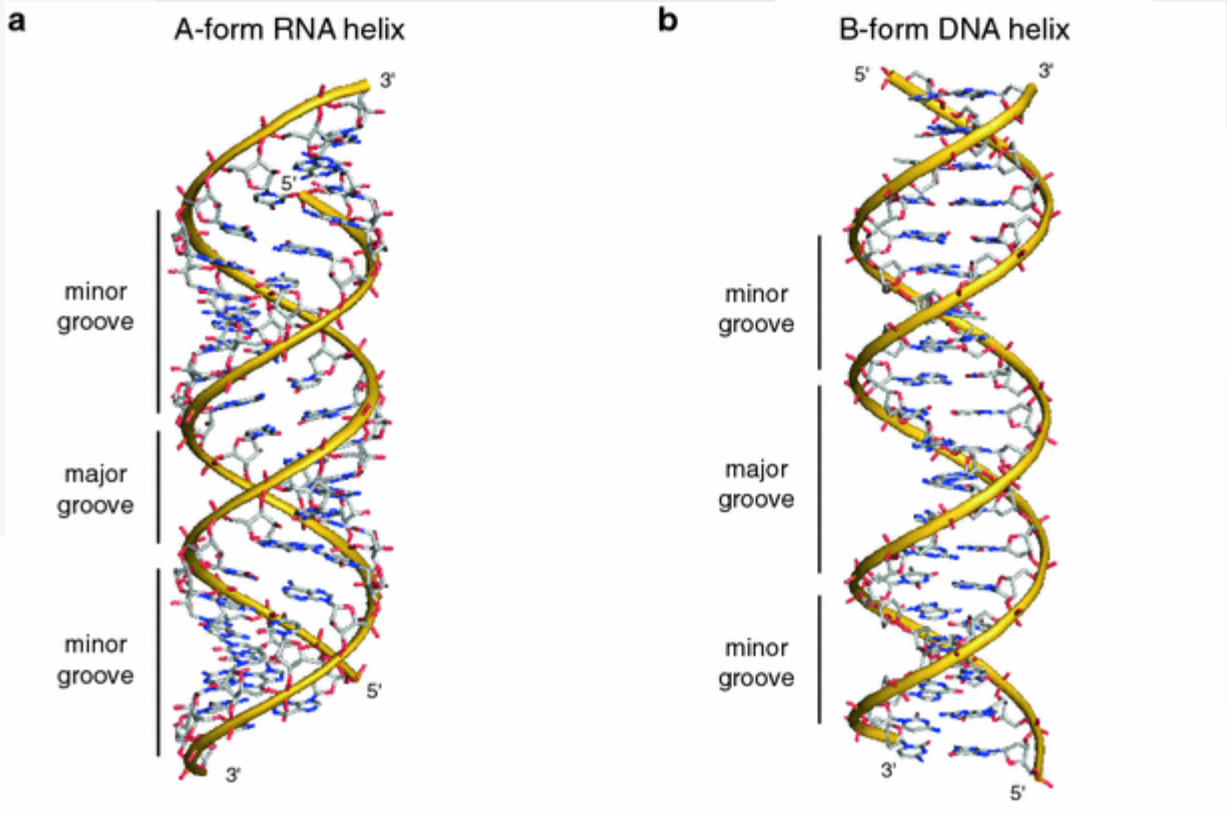
Innate immune pattern recognition receptors (PRRs) discriminate self from non-self

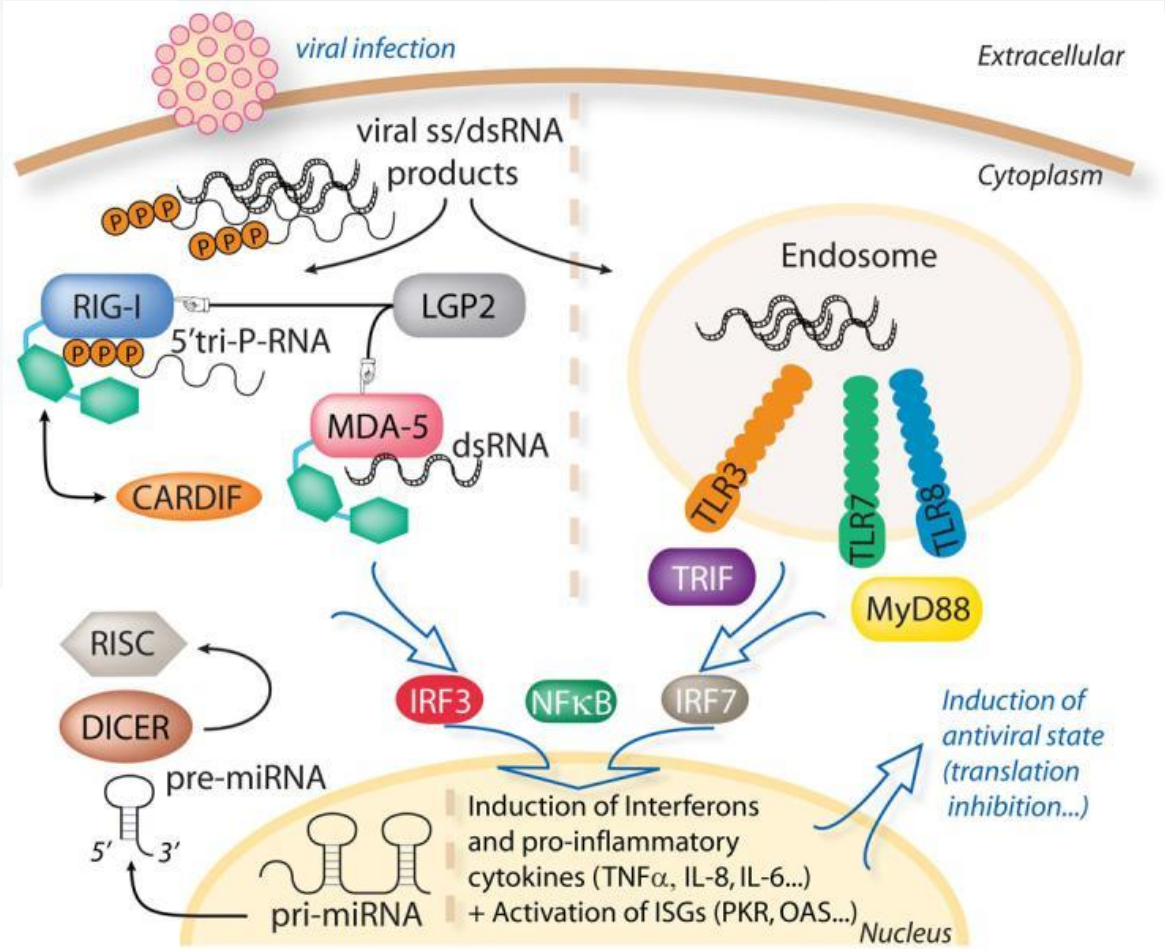
Charles Janeway

Polly Matzinger PRRs also react to damage or danger signals

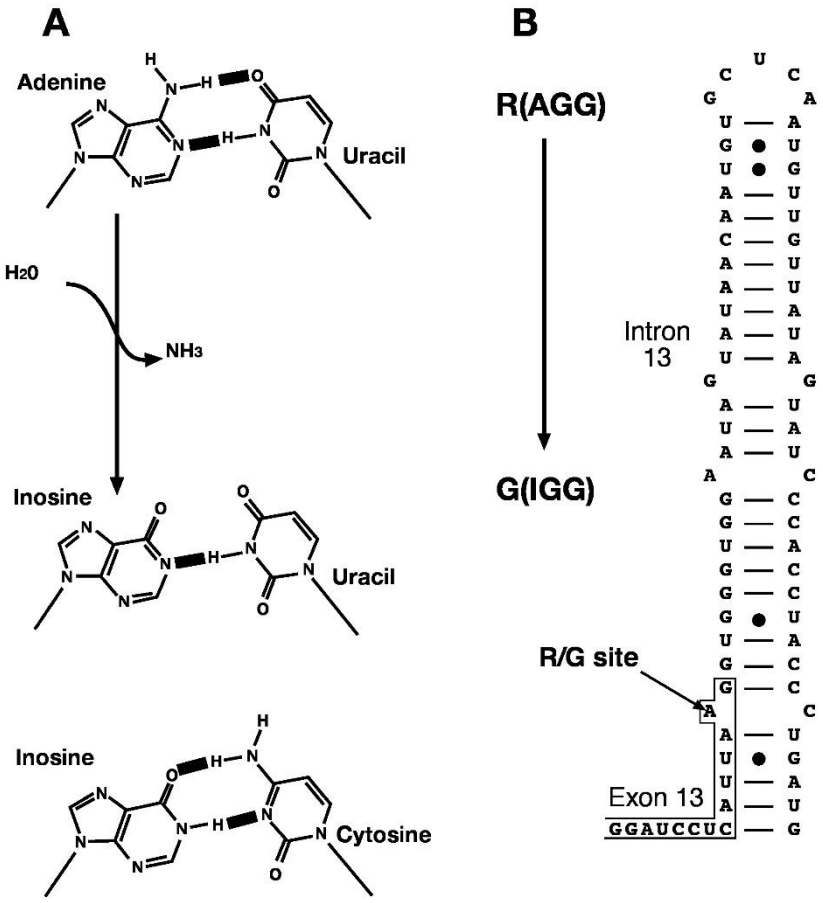
dsRNA versus DNA

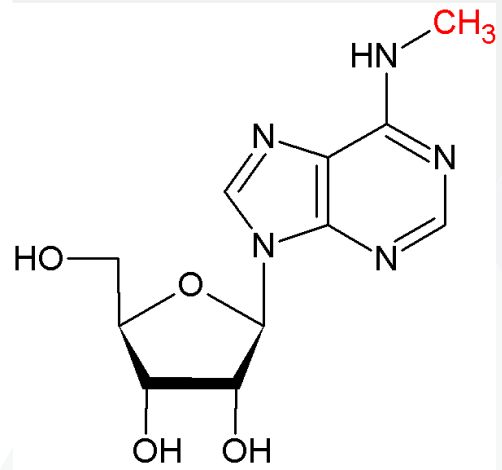


Cellular dsRNA is hazardous. Innate immune and RNAi trigger.

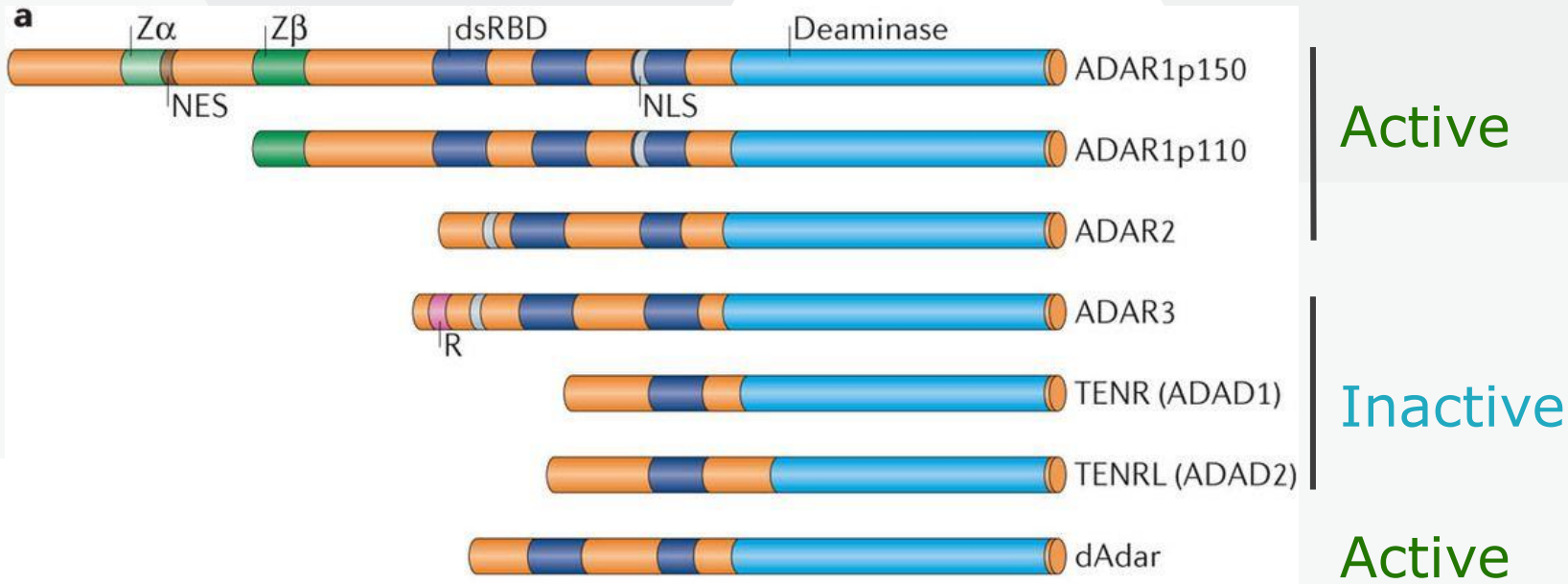


Conversion of adenosine to inosine change the encoded protein



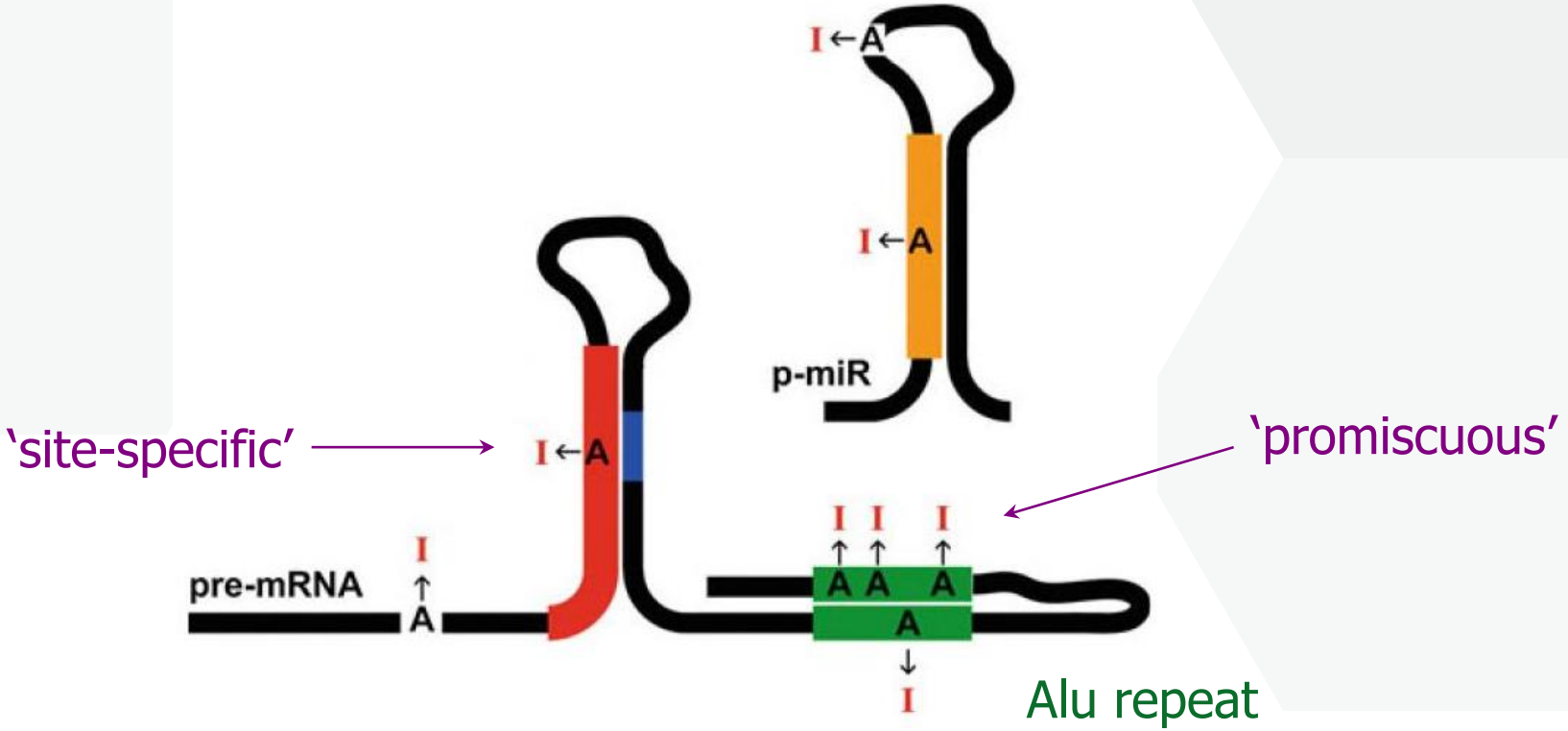


ADAR proteins in vertebrates and in *Drosophila*

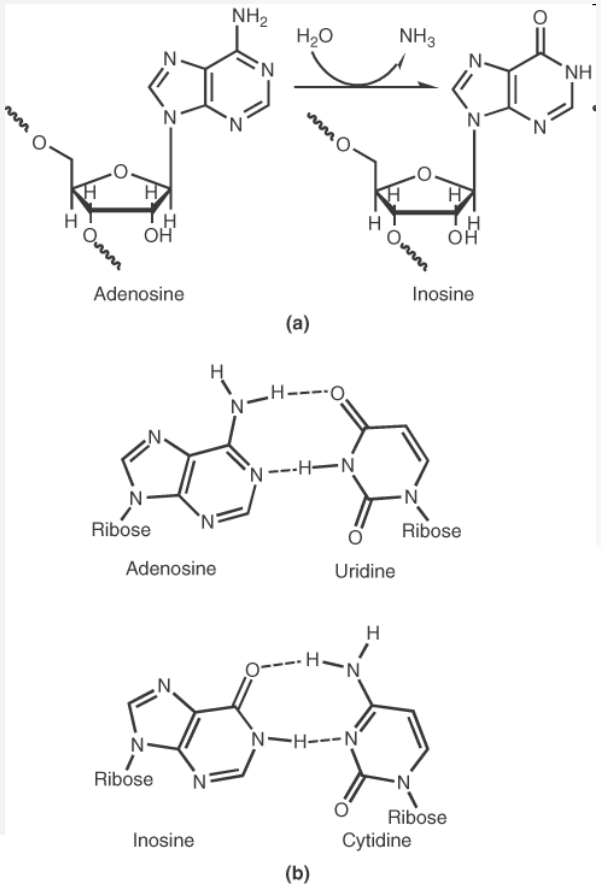


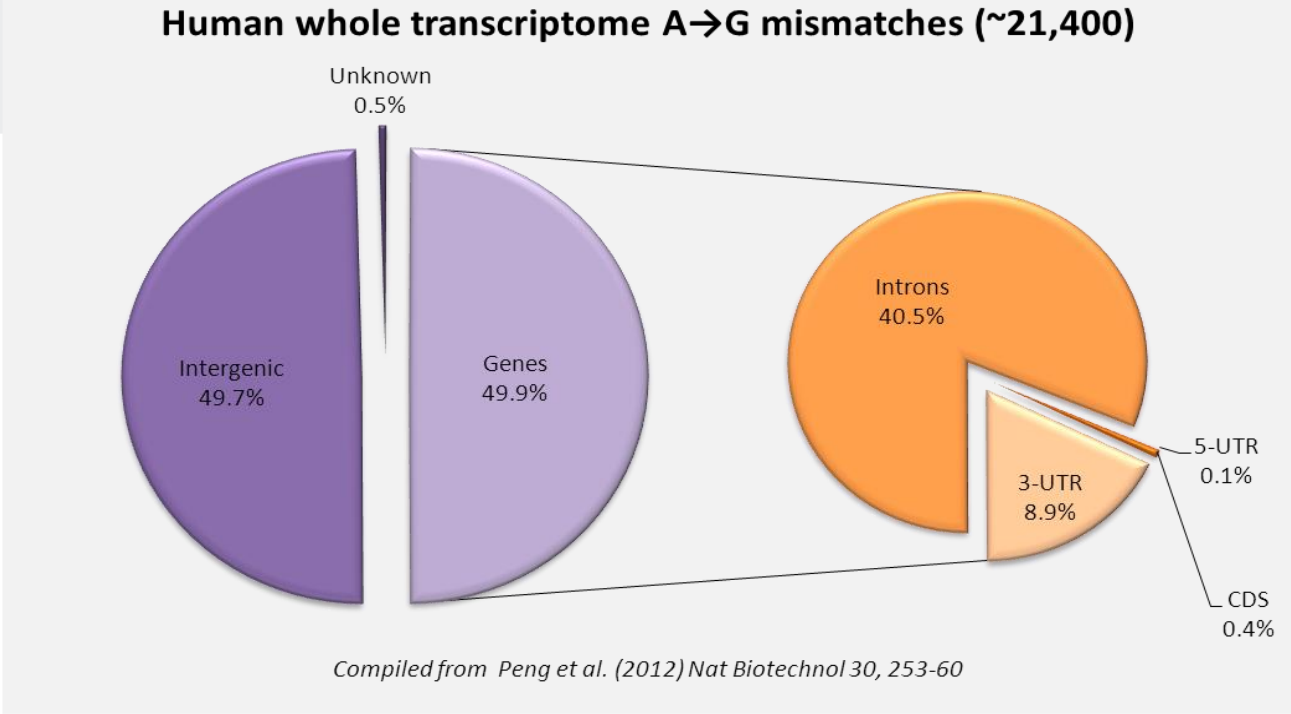
Nature Reviews | Molecular Cell Biology

Adenosine Deaminases acting on RNA (ADARs) edit A-to-I in dsRNA

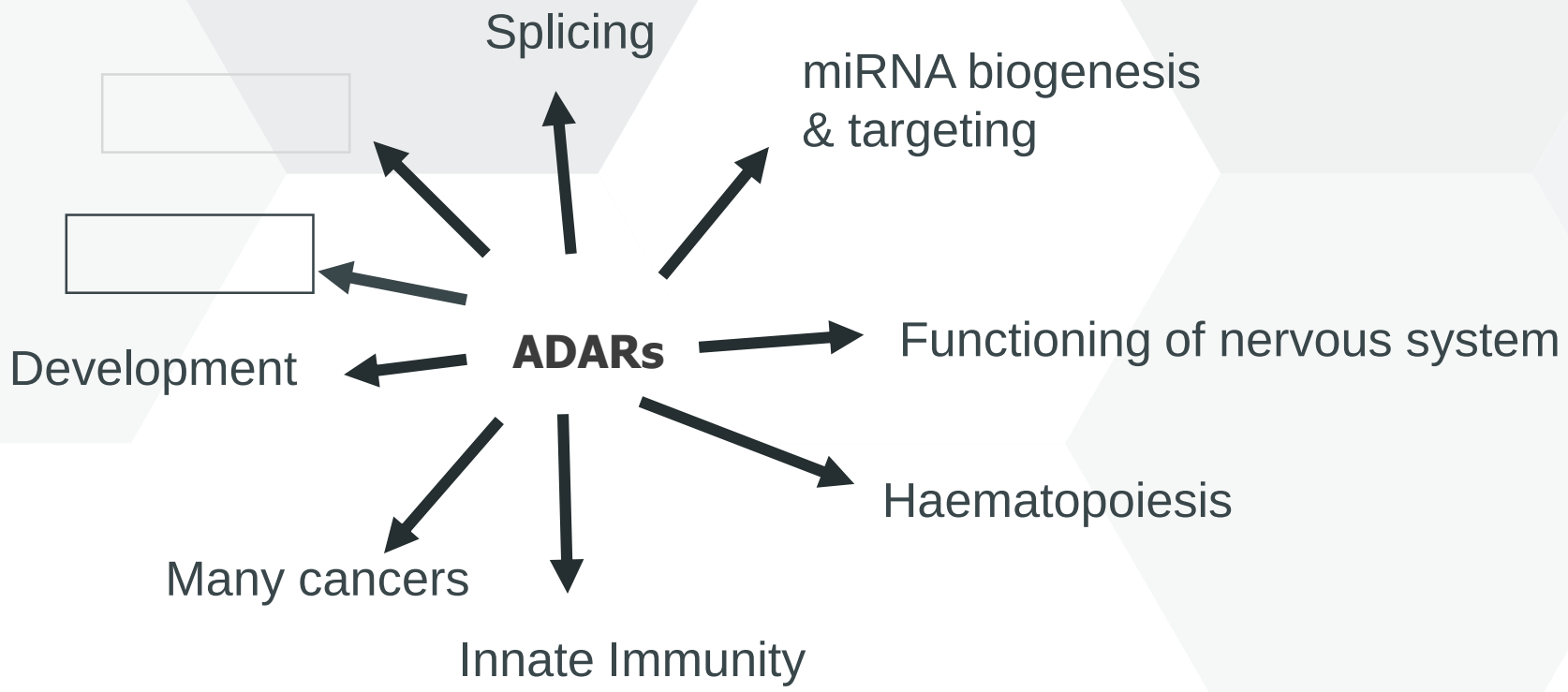


Prevalence of A-to-I editing





Biological roles of ADARs

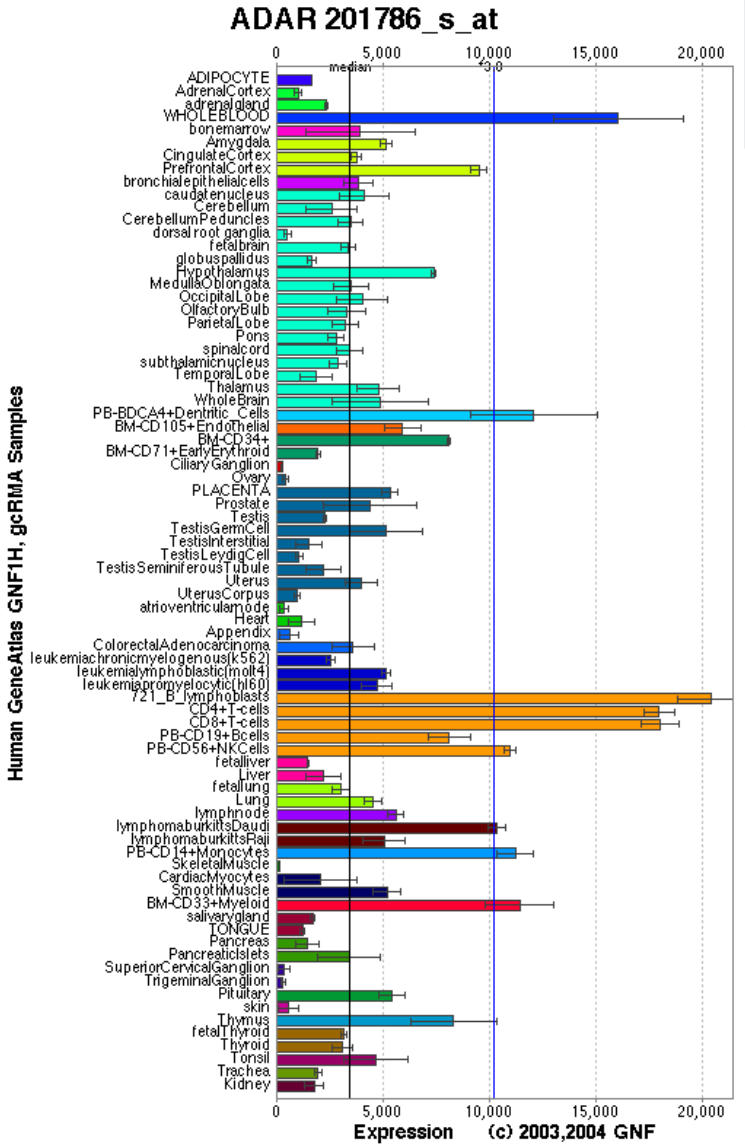


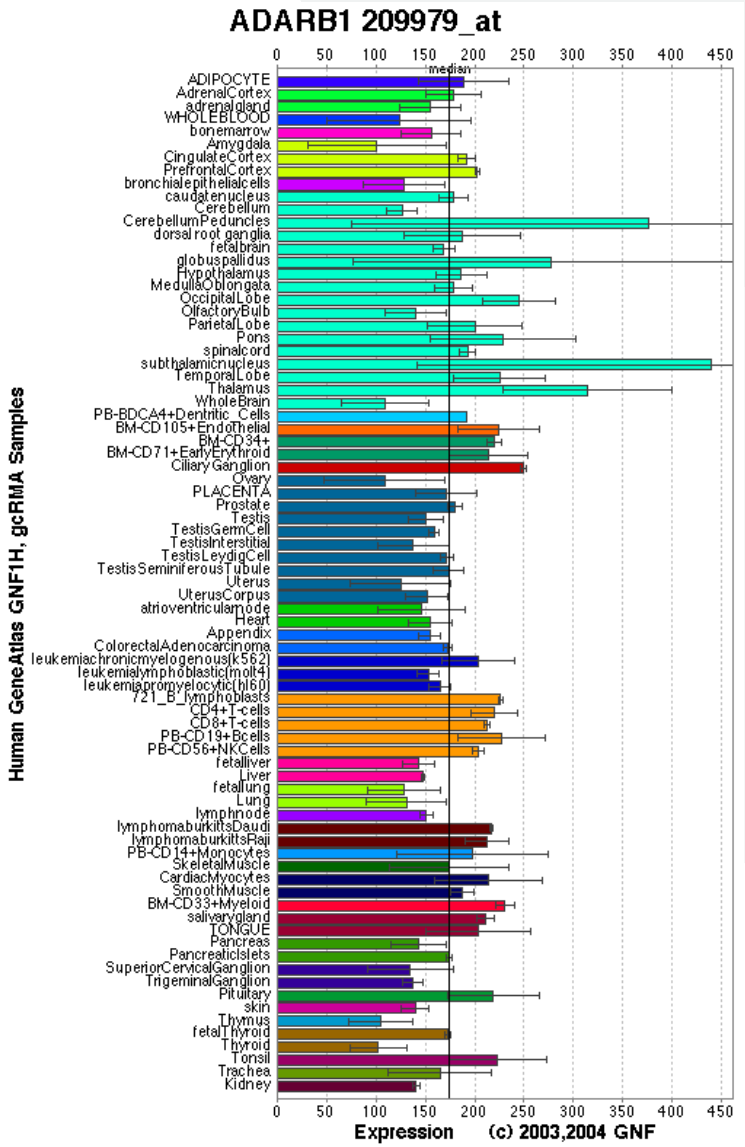
Editing dependent & independent roles of ADAR

Summary of ADAR mutant phenotypes in vertebrates and in *Drosophila*

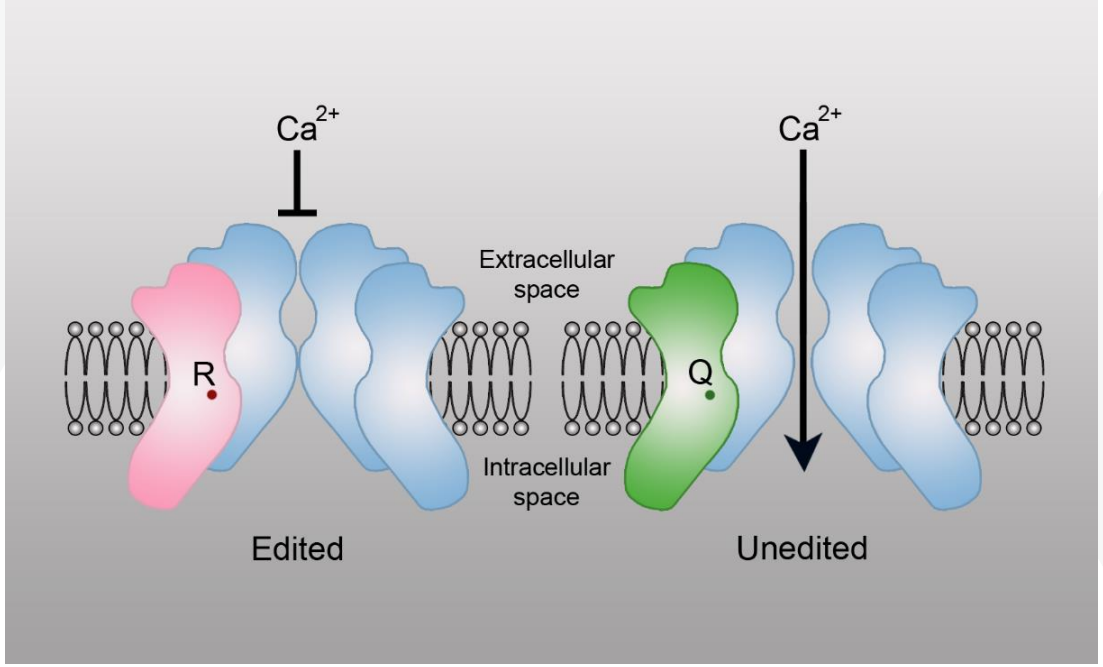
Mutated gene	*dAdar* $^{-/-}$	*Adar1* $^{-/-}$	*Adar2* $^{-/-}$
Tissue	CNS	Liver	Brain
Transcript	?	Alus elements	*Gria2 Q/R* (GluR2)
Phenotype	Behavior Locomotion Neurodegeneration	Hematopoiesis Innate immunity Death by E12.5	Seizures, death by P20

hADAR1 versus hADAR2 tissue expression





RNA editing of transcript encoding the GluR2 subunit of vertebrate glutamate AMPA receptors



ADARB1 mutations in childhood seizures EIMFS (epilepsy of infancy with migrating focal seizures)

- We have established a collaboration with Dr. Tiong Tan from the University of Melbourne and Dr. Mark Fitzgerald from Children's Hospital of Philadelphia, Dr. Riki Sukenik Halew, Meir Medical Center Israel,.
- hADAR2 variants were found in four patients (US patient now deceased) suffering with **microcephaly, severe intellectual disability and seizures**
- Australian patient: biallelic mutation in *ADAR2*; Lys367Asn, Thr498Ala
- US patient: homozygous mutation in *ADAR2*; Lys127Glu
- Israeli patient : homozygous mutation in *ADAR2;* Arg603Glu
- Iranian patients: homozygous mutation in *ADAR2*; Arg630Glu

  homozygous mutation in *ADAR2*; Ala722Val

Mutation in *KCNT1* also result in EIMSF

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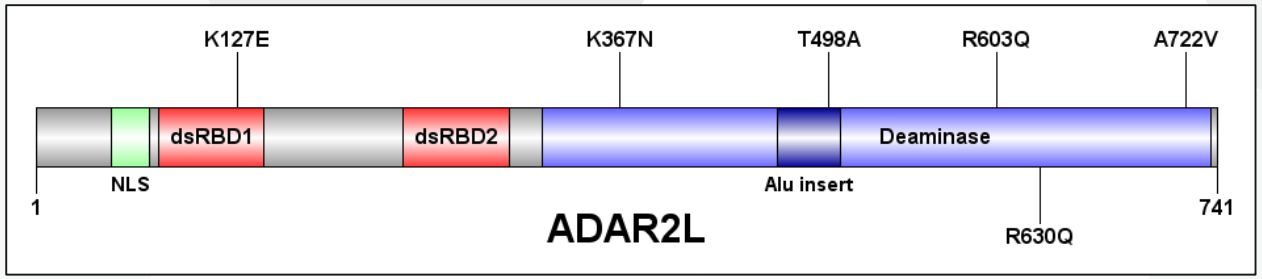
Tan *et al.* Am J Hum Genet. 2020, Maroofian et *al.* J Med Genet. 2021

Patients with *ADARB1* variants

Tan *et al.* Am J Hum Genet. 2020,

Maroofian et *al.* J Med Genet. 2021

Locations of ADAR2 mutations in *ADAR2* Variants

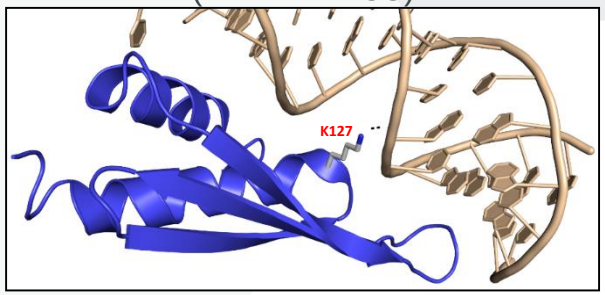


Tan *et al.* Am J Hum Genet. 2020,

Maroofian et *al.* J Med Genet. 2021

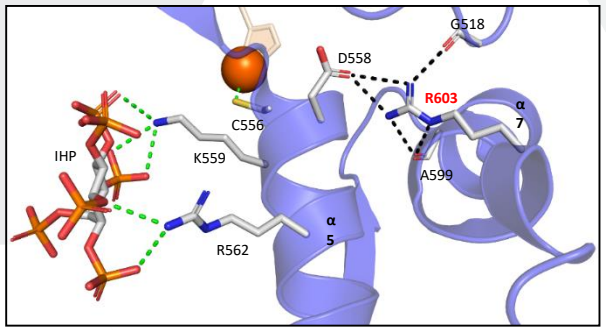
Location of Variants

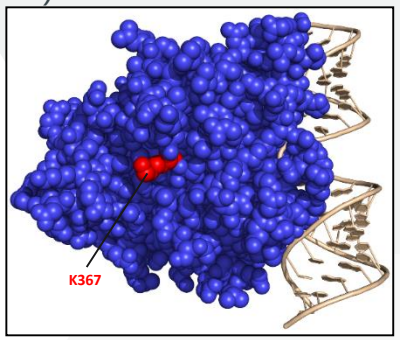
ADAR2 dsRBD1
(PDB ID: 2L3C)

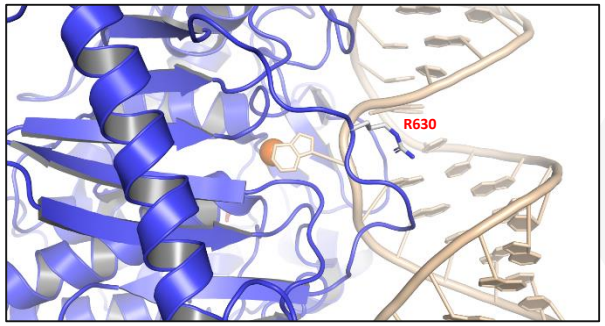


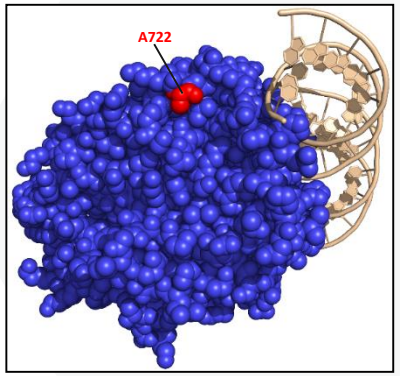
Stefl R. et al., Cell (2010) 143, 225-237.

ADAR2 deaminase domain
(PDB ID: 5ED1)









Matthews M. et al., Nature Structural & Molecular Biology (2016) 23, 426–433.

- dsRBD1 or deaminase domain in **blue**
- dsRNA substrate in **wheat**, Zn^{2+} in **orange**

Tan *et al.* Am J Hum Genet. 2020,

Maroofian et *al.* J Med Genet. 2021

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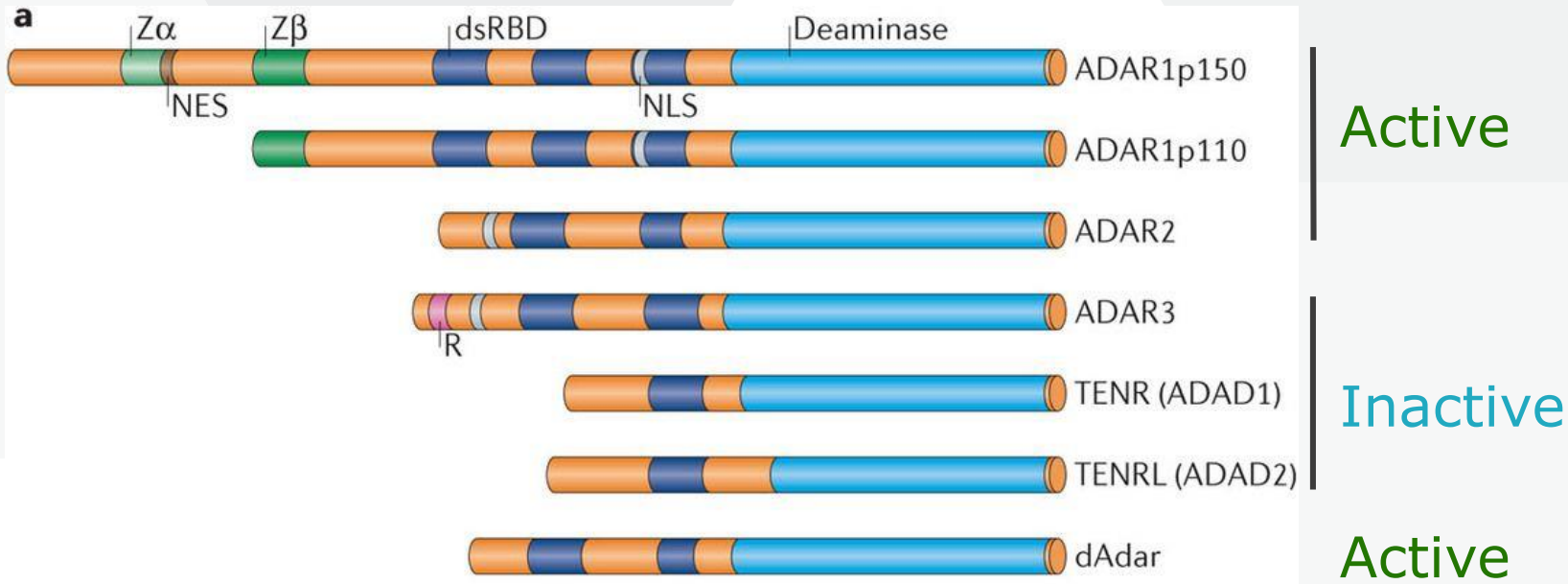
ADAR proteins in vertebrates and in *Drosophila*



Nature Reviews | Molecular Cell Biology

nature genetics

Mutations in *ADAR1* cause Aicardi-Goutières syndrome associated with a type I interferon signature

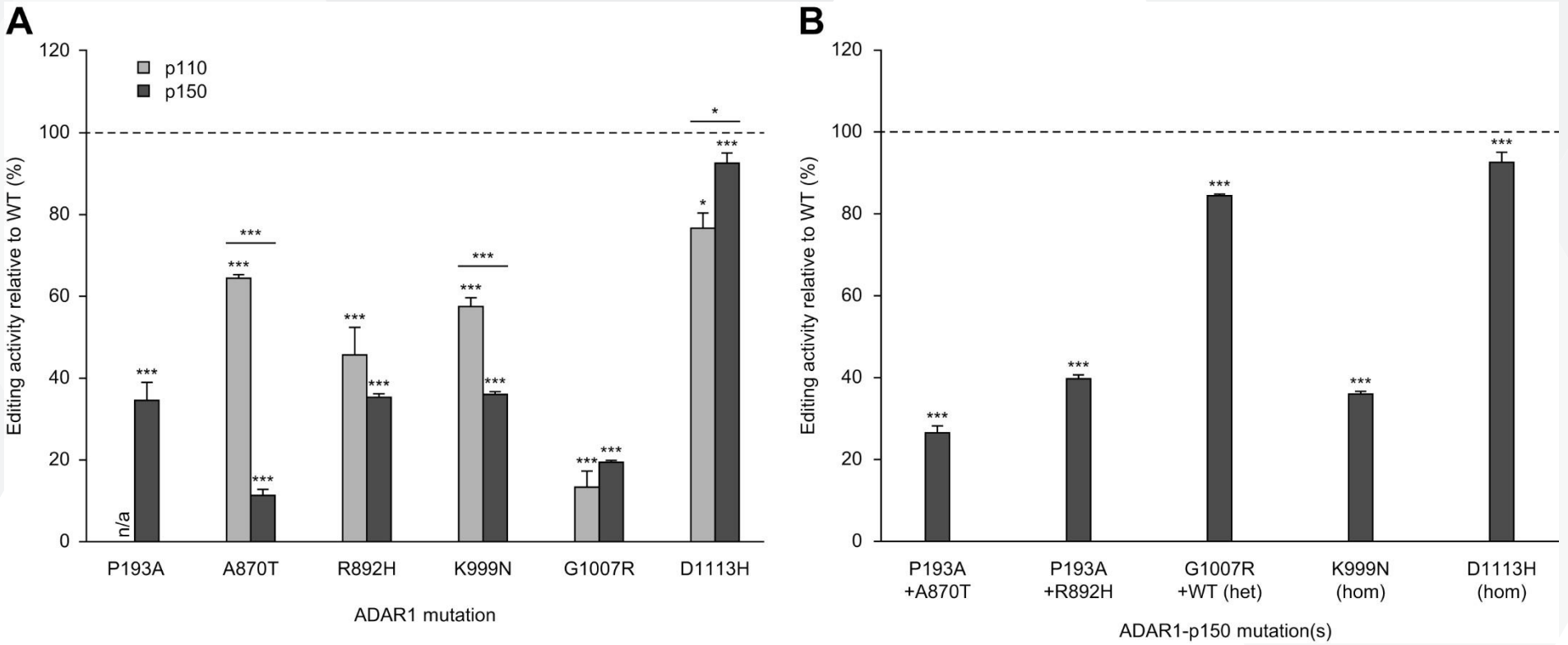
Gillian I Rice[1], Paul R Kasher[1], Gabriella M A Forte[1], Niamh M Mannion[2], Sam M Greenwood[2], Marcin Szynkiewicz[1], Jonathan E Dickerson[1], Sanjeev S Bhaskar[1], Massimiliano Zampini[1], Tracy A Briggs[1], Emma M Jenkinson[1], Carlos A Bacino[3], Roberta Battini[4], Enrico Bertini[5], Paul A Brogan[6], Louise A Brueton[7], Marialuisa Carpanelli[8], Corinne De Laet[9], Pascale de Lonlay[10], Mireia del Toro[11], Isabelle Desguerre[12], Elisa Fazzi[13], Àngels Garcia-Cazorla[14,15], Arvid Heiberg[16], Masakazu Kawaguchi[17], Ram Kumar[18], Jean-Pierre S-M Lin[19], Charles M Lourenco[20], Alison M Male[21], Wilson Marques Jr[20], Cyril Mignot[22–24], Ivana Olivieri[25], Simona Orcesi[25], Prab Prabhakar[26], Magnhild Rasmussen[27], Robert A Robinson[26], Flore Rozenberg[28], Johanna L Schmidt[29], Katharina Steindl[30], Tiong Y Tan[31], William G van der Merwe[32], Adeline Vanderver[29], Grace Vassallo[33], Emma L Wakeling[34], Evangeline Wassmer[35], Elizabeth Whittaker[36], John H Livingston[37], Pierre Lebon[28], Tamio Suzuki[17], Paul J McLaughlin[38], Liam P Keegan[2], Mary A O'Connell[2], Simon C Lovell[39] & Yanick J Crow[1]

Adenosine deaminases acting on RNA (ADARs) catalyze the hydrolytic deamination of adenosine to inosine in double-stranded RNA (dsRNA) and thereby potentially alter the information content and structure of cellular RNAs. Notably, although the overwhelming majority of such editing events occur in transcripts derived from Alu repeat elements, the biological function of non-coding RNA editing remains uncertain. Here, we show that mutations in *ADAR1* (also known as *ADAR*) cause the autoimmune disorder Aicardi-Goutières syndrome (AGS). As in *Adar1*-null mice, the human disease state is associated with upregulation of interferon-stimulated genes, indicating a possible role for ADAR1 as a suppressor of type I interferon signaling. Considering recent insights derived from the study of other AGS-related proteins, we speculate that ADAR1 may limit the cytoplasmic accumulation of the dsRNA generated from genomic repetitive elements.

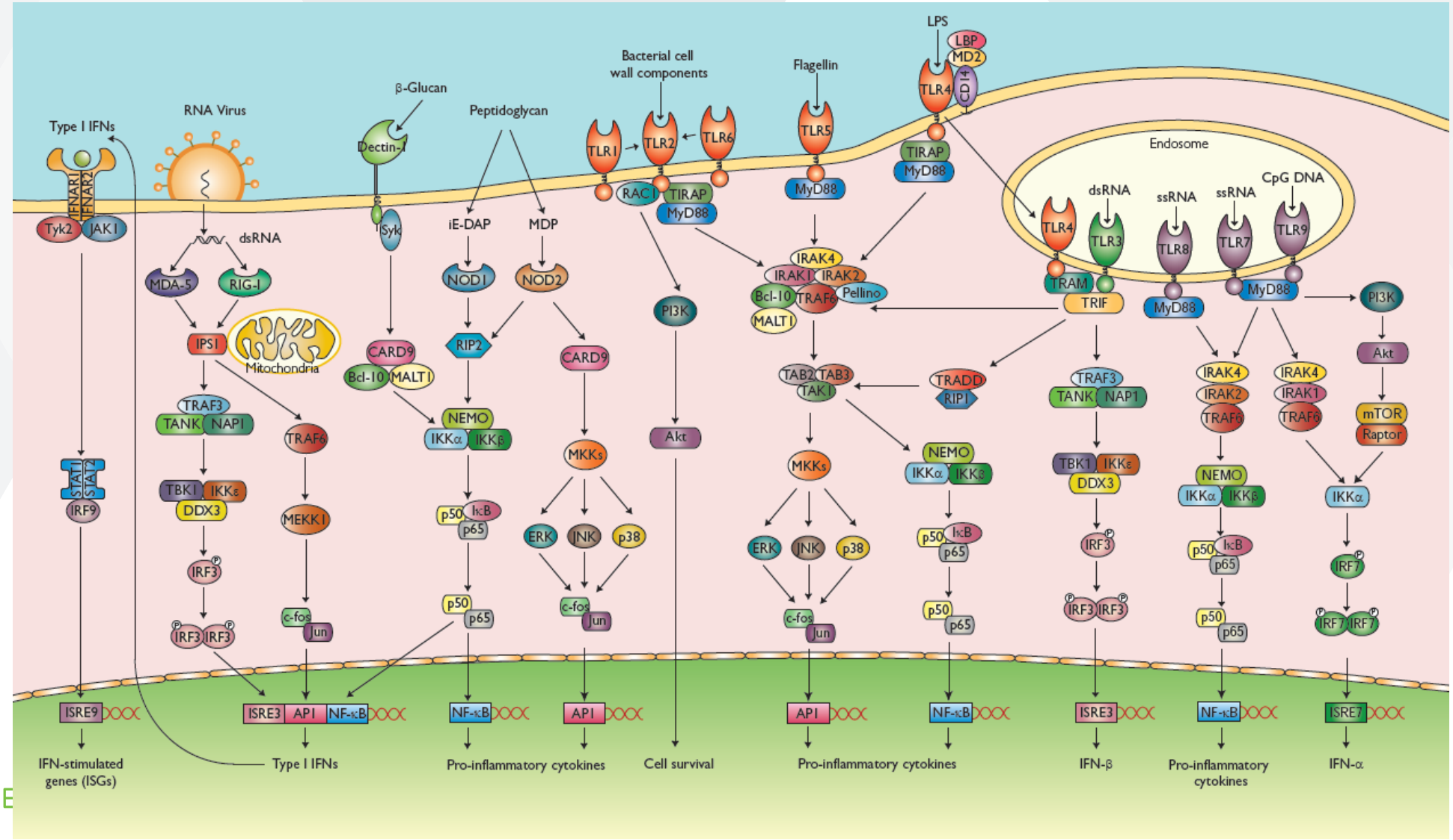
autosomal recessive trait[7], rare examples of disease due to *de novo* dominant mutations in *TREX1* have been reported[8–10].

Studies of the function of TREX1 have delineated a cell-intrinsic mechanism for the initiation of an autoimmune response by interferon (IFN)-stimulatory nucleic acid[11,12], begging the question of the source of nucleic acid inducing the type I IFN–mediated immune disturbance in AGS. In this regard, it has been shown that TREX1 can metabolize reverse-transcribed DNA and that single-stranded DNA derived from endogenous retroelements accumulates in TREX1-deficient cells[11]. On a related note, TREX1 (ref. 13), SAMHD1 (refs. 14–16) and RNase H2 (ref. 17) have been implicated in the metabolism of the (exogenous) retrovirus HIV-1. Perhaps most notably, a recent study showed rescue of the lethal inflammatory TREX1-null mouse phenotype by a combination of reverse transcriptase inhibitors (antiretroviral therapy as used to treat HIV-1)[18], suggesting that the accumulation of cytosolic DNA in TREX1-null cells can be ameliorated by inhibiting endogenous retroelement cycling.

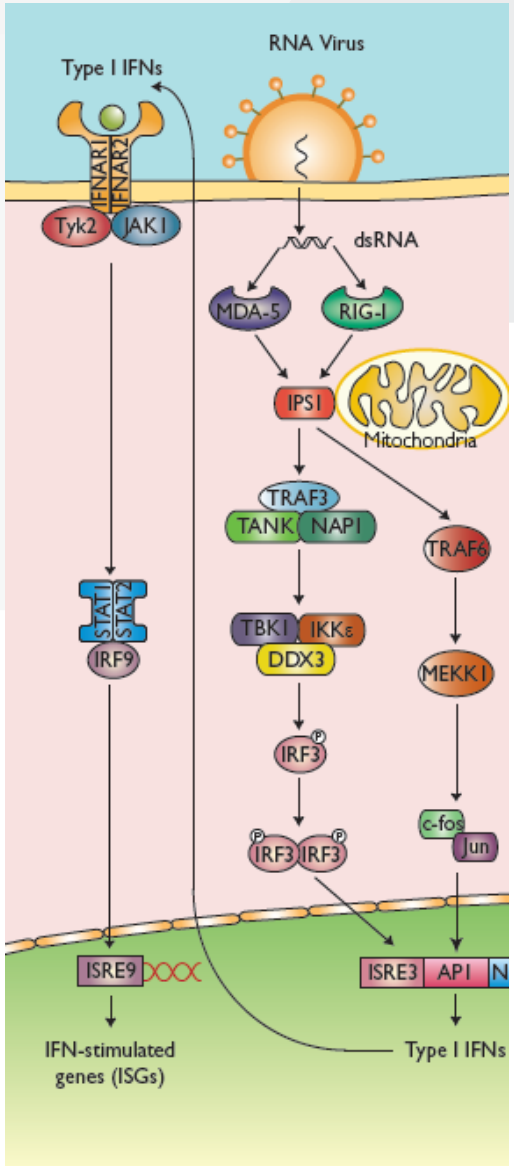
Combination of mutations in *ADAR1* as found in AGS patients.



Innate immune system
Pattern Recognition Receptor signalling



Rescue of *Adar1* lethality by preventing innate immune sensing of intracellular dsRNA



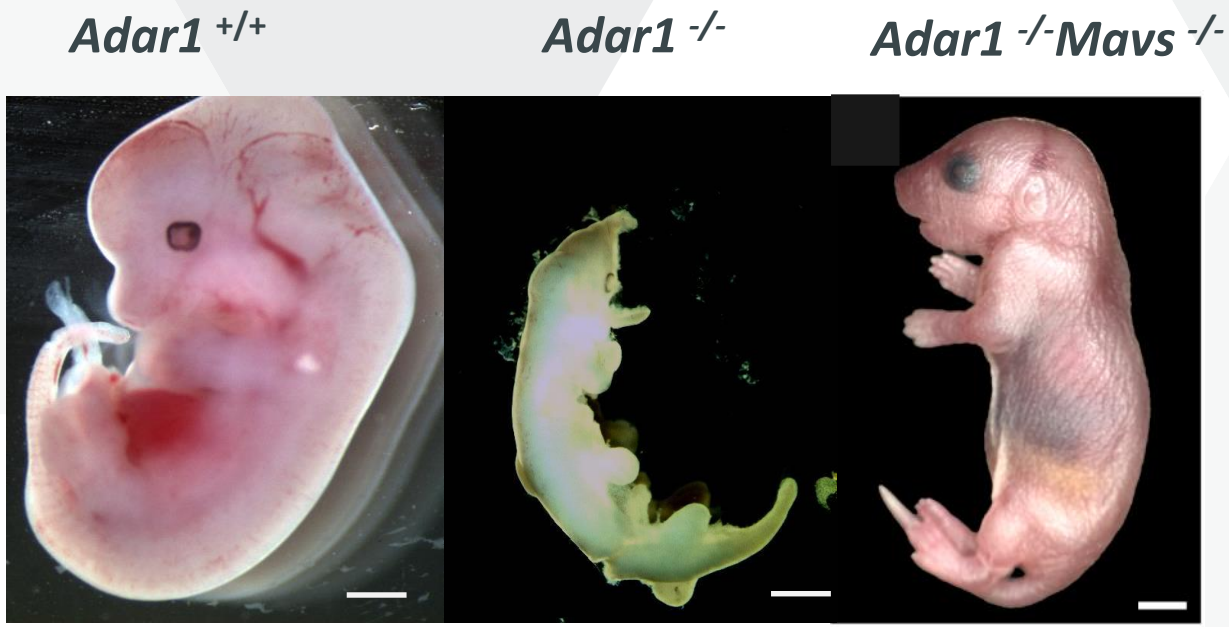
Mavs/IPS-1 knockout prevents all known signalling in this pathway.

Mannion, 2014; complete null, death E12.5. Double mutant with *Mavs* dies at birth.

Liddicot 2015; inactive mutant, death by E14.5. Double mutant with *Mda5* has *no phenotype*.

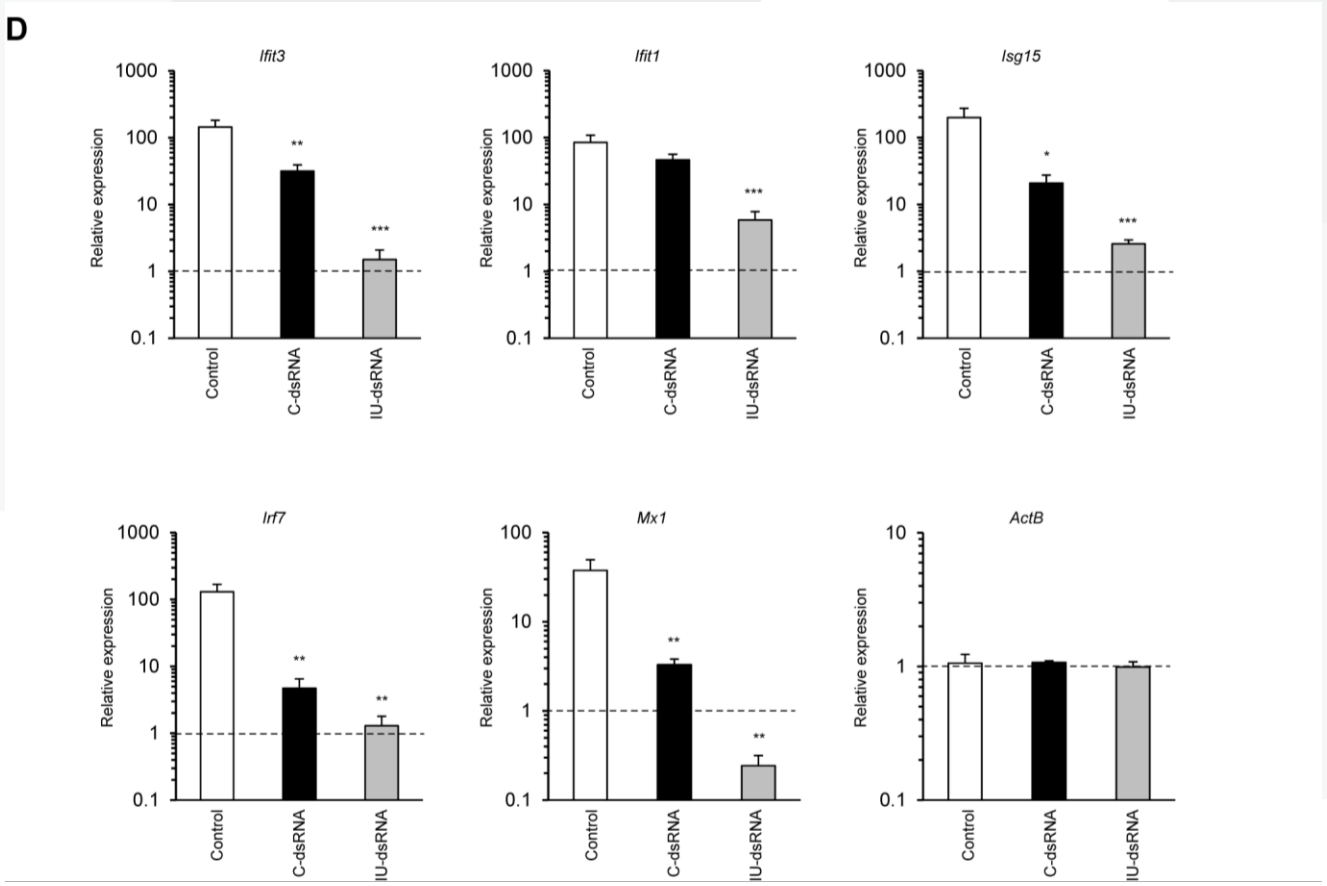
Sun Hur 2018 demonstrated that transcripts encoding inverted Alus activate MDA5

Mavs $^{-/-}$ rescue of *Adar1* $^{-/-}$ to birth

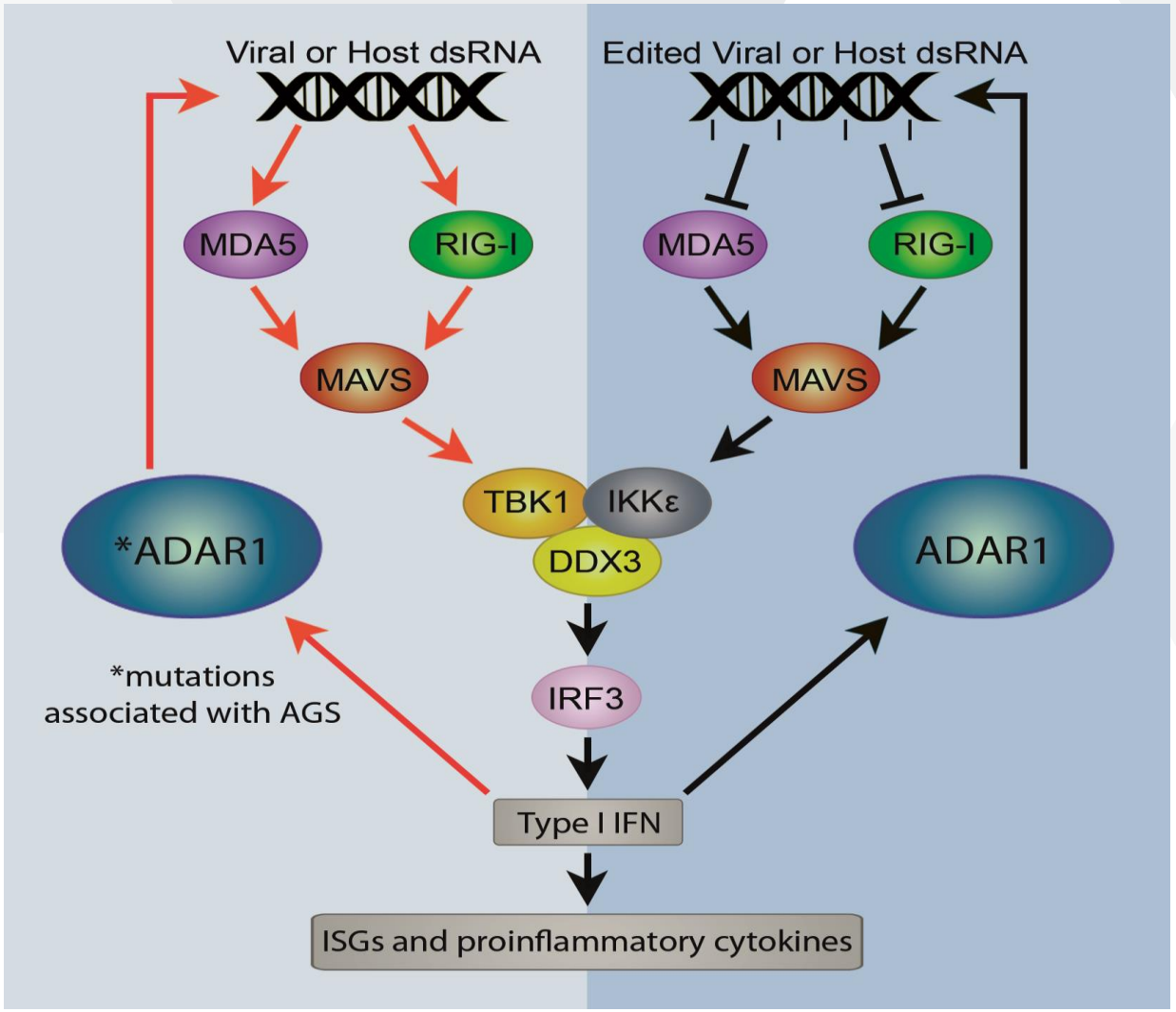


Mannion *et al*. Cell Reports 2014

IU-dsRNA oligo reduces innate immune response in stressed *Adar1; p53* MEF

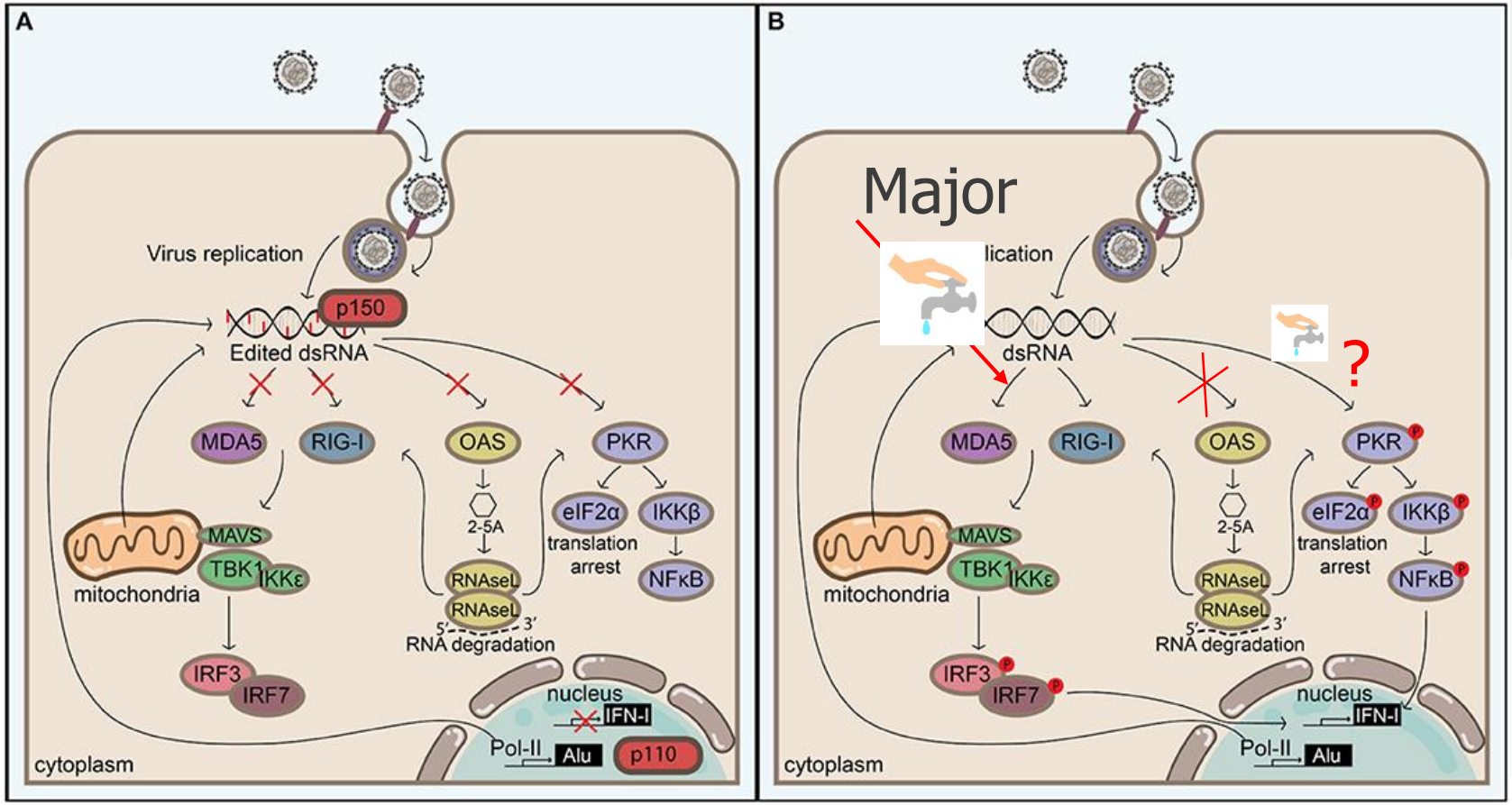


Inosine in RNA helps discriminate self from non-self



Adar1$^{-/-}$;*Mavs*$^{-/-}$ (complete null)

Pkr alone does not recue *Adar*



Adapted from Lamers *et al.* Front. Immunol., 25 July 2019

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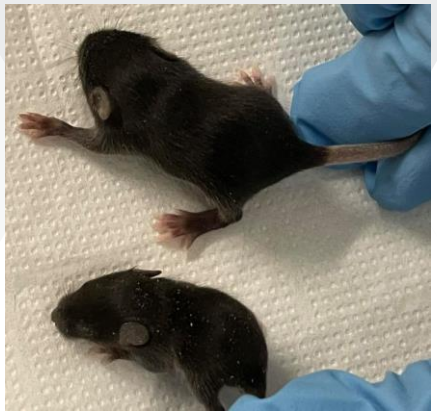
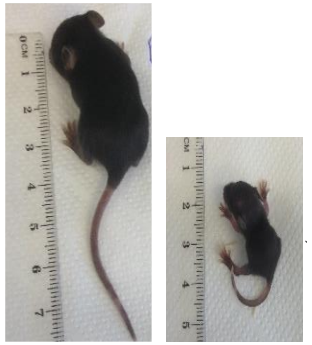
PKR deletion phenotype



Eif2ak2;Adar;Mavs 18days



Adar;Mavs 15d

***Eif2ak2* deletion in *Adar,Mavs* rescues even when it is heterozygous!**

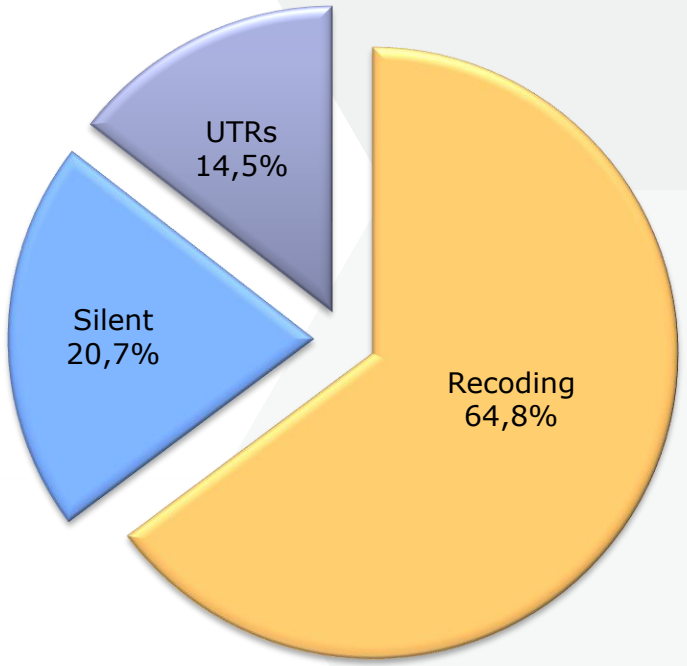
What is the inactive role of ADARs?

A → I editing in *Drosophila melanogaster*

Poly(A)$^+$ RNA-Seq: 972 sites in 597 transcripts

630 alter amino acids
201 silent
141 within UTRs

~20% of endogenous small RNAs bound to AGO2 are edited

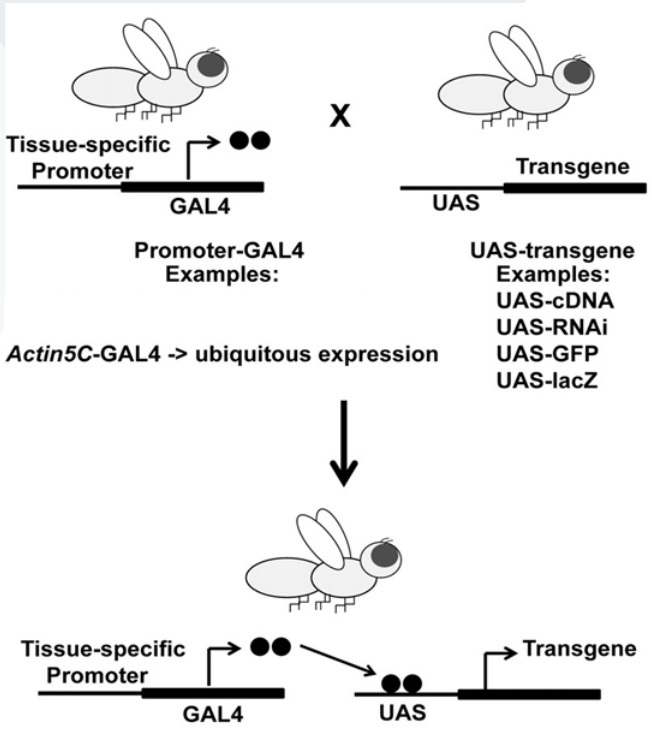


Graveley *et al.* Nature 2011

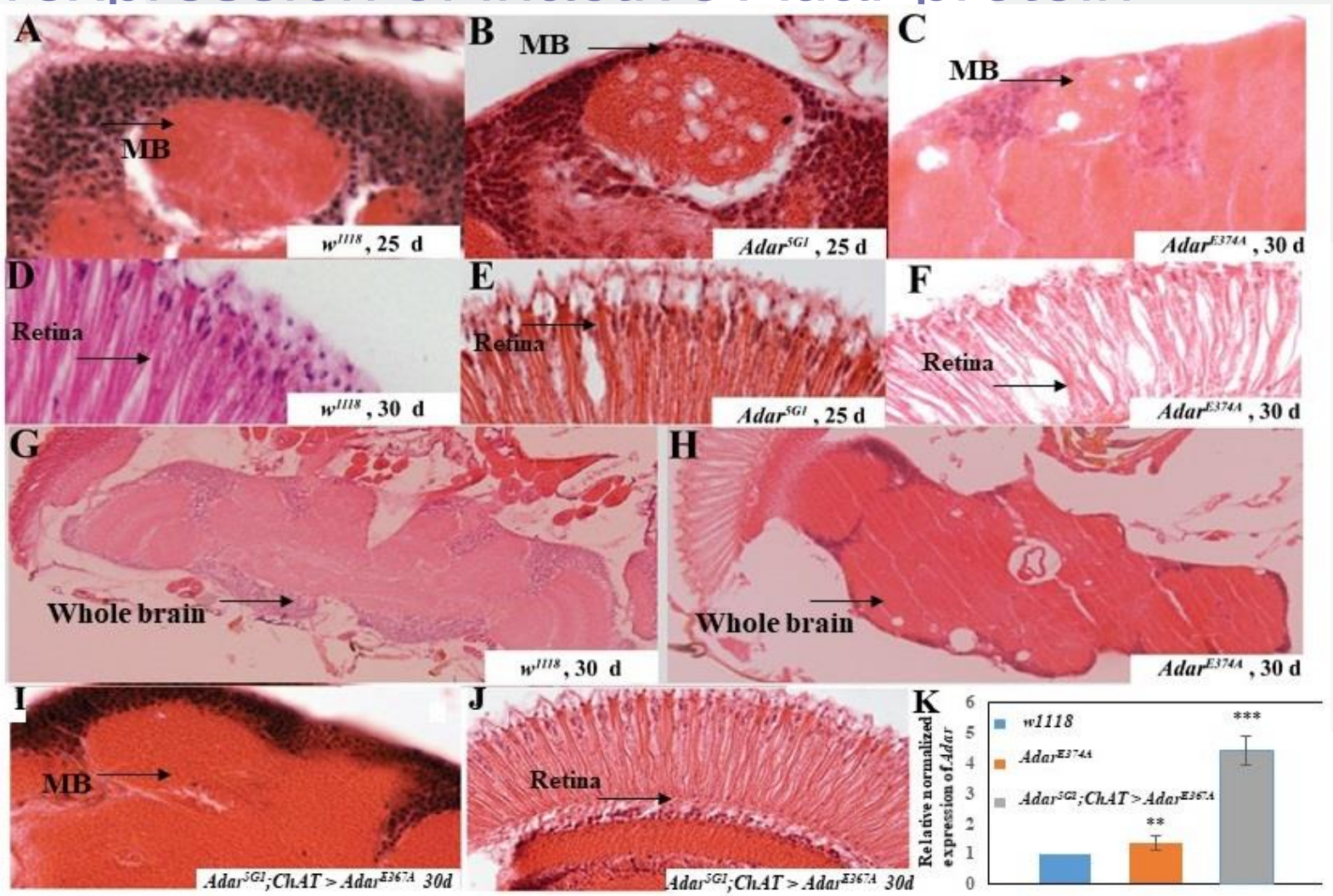
High RNA editing in neurotransmitter receptor subunits.

Name	Protein function	Number of sites	Functional consequence*	• % editing at each site
	Voltage-gated ion channels			
DSCl	Na^+ channel	1	+++	• 50%
Ca-alpha1T	Ca^{2+} channel	1	+	• 30%
DmCa1D	Ca^{2+} channel	5	++	• 30, 100, 95, 95, 100%
$\alpha_2\delta$	Ca^{2+} channel accessory subunit	3	+	• 80, 50, 50%
Shaker (*Sh*)	K^+ channel	6	+++	• 10, 10, 50, 50, 80, 50%
ether-a-go-go (*eag*)	K^+ channel	6	+++	• 50, 100, 20, 90, 10, 80%
slowpoke (*slo*)	K^+ channel	2	+	• 90, 90%
	Synaptic release machinery			
Synaptotagmin (*syt*)	Ca^{2+} sensor	4	+++	• 5, 50, 50, 100%
Dunc-13	SNARE binding	1	++	• 40%
Stoned B (*stnB*)	?	1	+	• 90%
complexin (*cpx*)	SNARE protein	3	?	• 50, 20, 30%
lap	Adaptor protein	1	?	• 10%
	Ligand-gated ion channels			
Dα5	nAChRα subunit	7	+++	• 80, 80, 100, 50, 30, 60%
ARD	nAChRβ subunit	4	?	• 50, 90, 50, 100%
SBD	nAChRβ subunit	2	+++	• 30, 20%
Resistance to dieldrin (*Rdl*)	GABA-receptor	6	+++	• 15, 80, 90, 90, 20, 10%

GAL4-UAS system



Neurodegeneration is less severe in inactive *Adar* mutant and can be rescued by overexpression of inactive Adar protein



Conclusions

- *ADARs are important dsRNA binding protein so the expression level of the protein is critical.*

- *ADARs role in innate immune response is maintained despite the evolution of very different pathways.*

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MARIE CURIE ACTIONS

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