PROPEL 101: Molecular mechanisms

10/26/23

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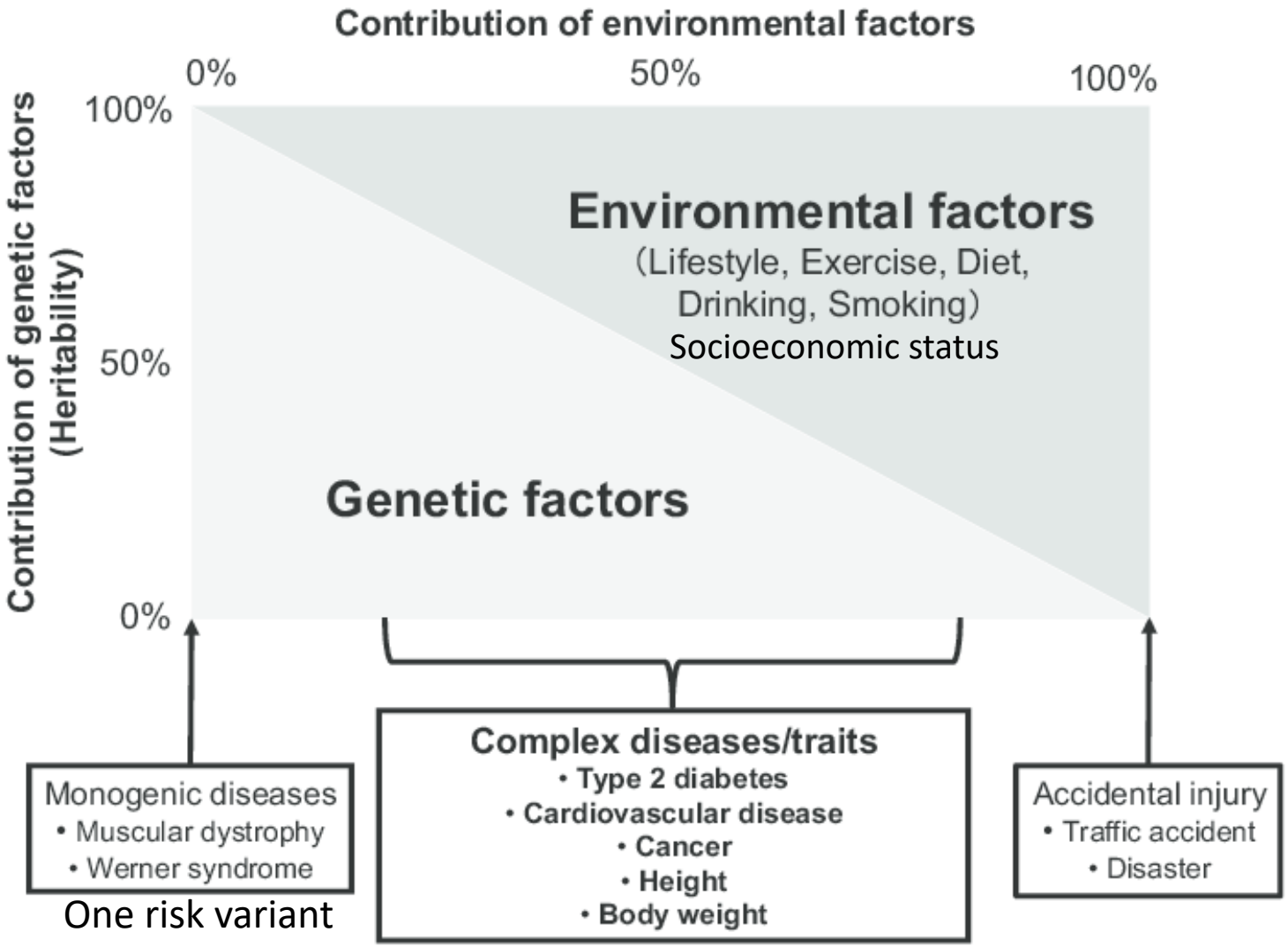
Recommendations for approaching the reading materials

- For the Supplemental material:
  - Not about memorizing/learning ALL the different mutations, etc.
  - Meant to help appreciate the scope of just how many ways genetic variation, acting through RNA and protein functionally, can contribute to disease
  - Pick a couple sections that sound most interesting and do a deep dive on those. But don’t worry about absorbing the details of all of it
- For the journal club
  - Primary goal to translate the 6 major mechanism stages we’re about to discuss to describe the specific approach/details of the paper

Why do we perform scientific research?

- In your previous two sessions, you've learned about some tools we have at our disposal to perform research.
- But why do we do it?

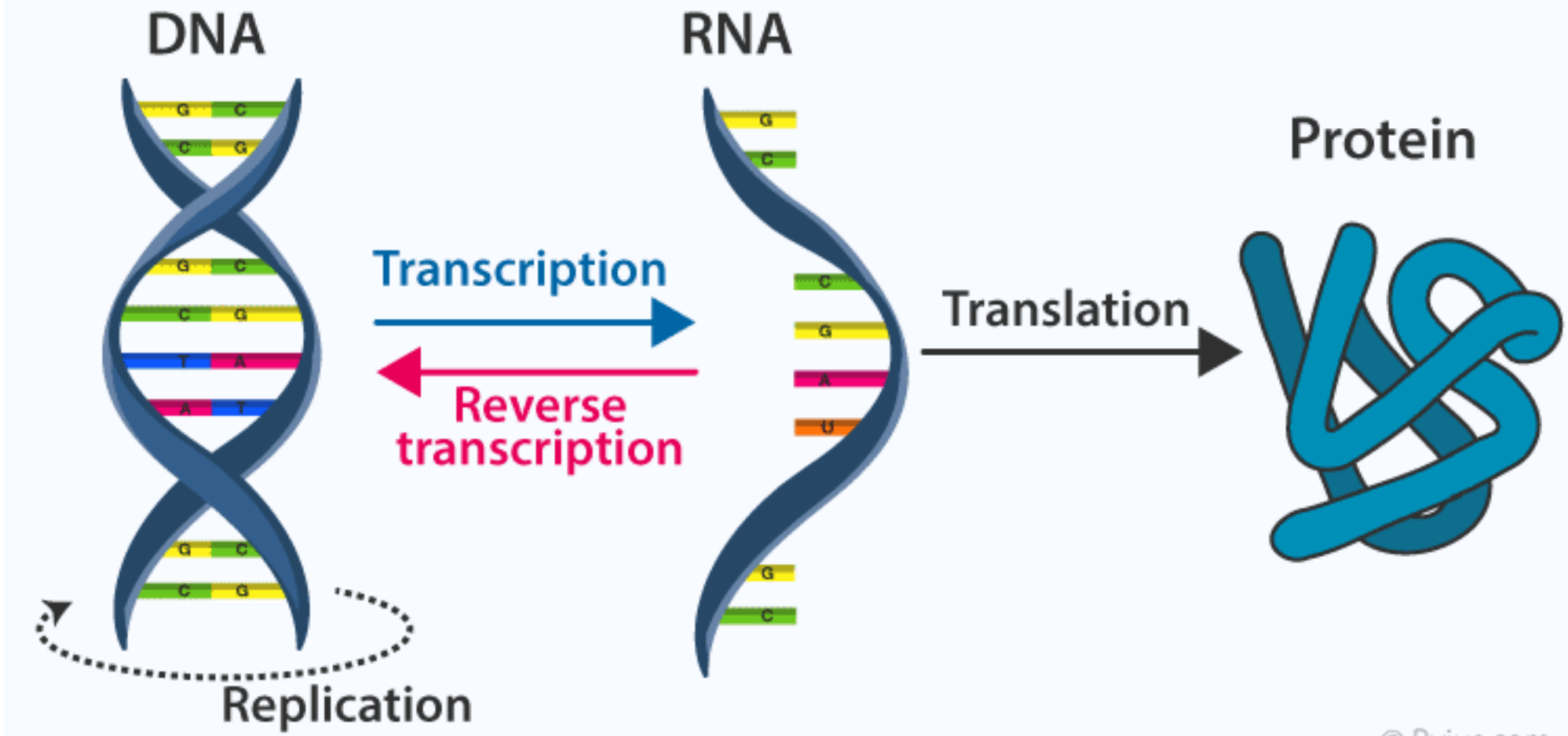
What is disease?

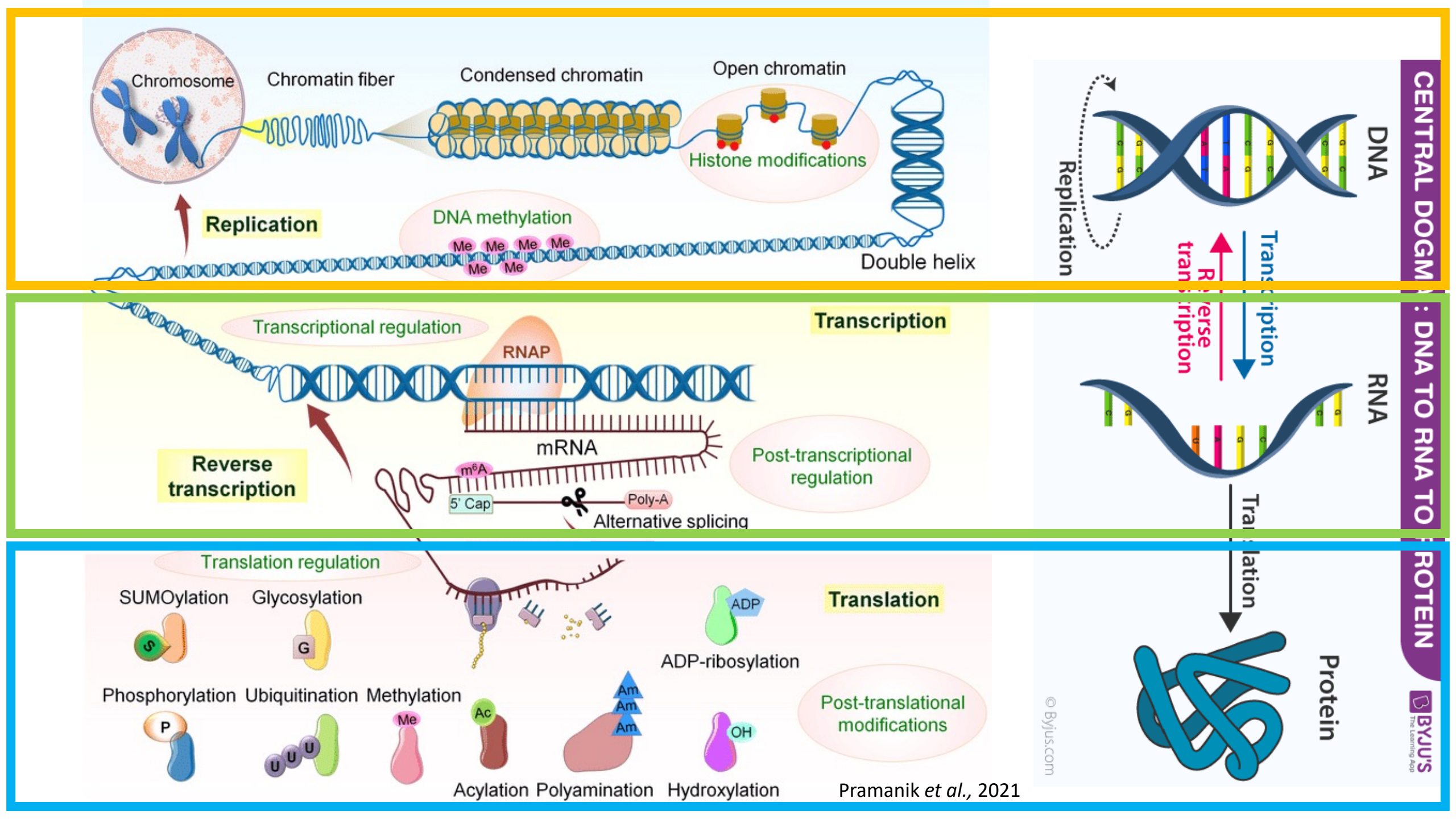


Tanisawa et al., 2016

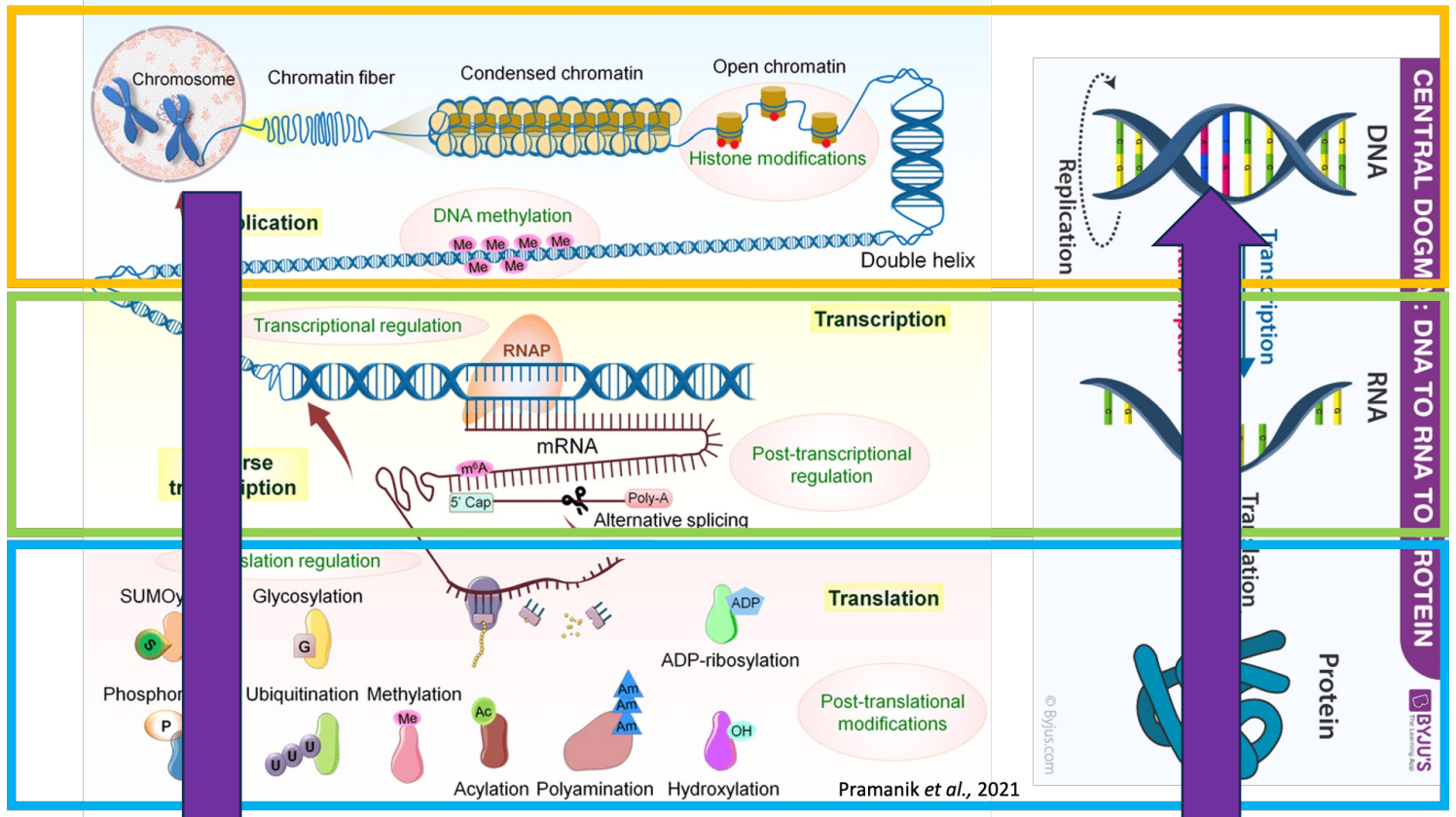
What is a “molecular mechanism”?

CENTRAL DOGMA : DNA TO RNA TO PROTEIN





Pramanik *et al.,* 2021



Cell function

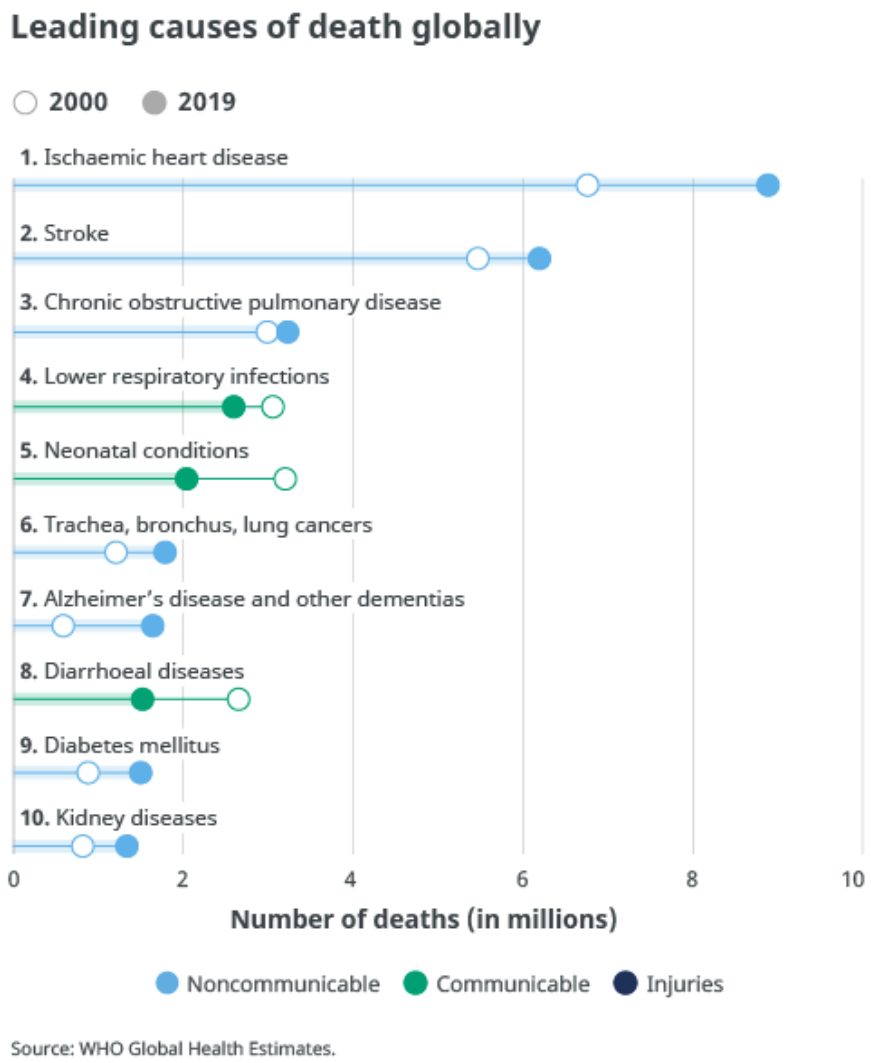
Organ/tissue function

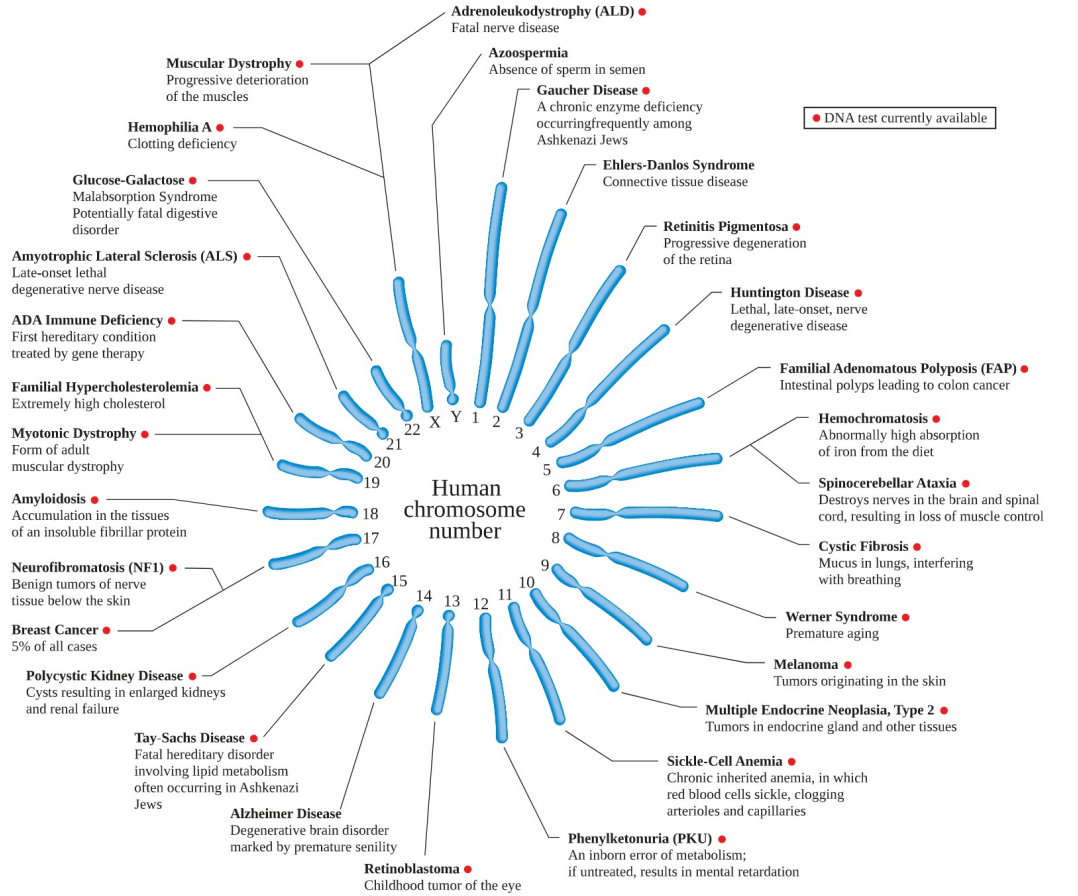
Systemic/organismal function

How to establish a convincing molecular mechanism

- *The journal club paper follows this construct, so if you can isolate the different stages of their analysis and associate them with each of these steps, it will make understanding and presenting it a little easier*

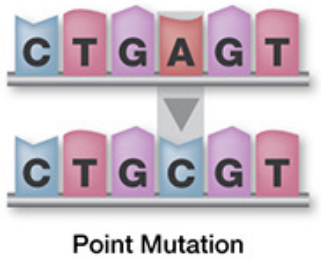
1. Choose your disease/phenotype of interest





Wikipedia

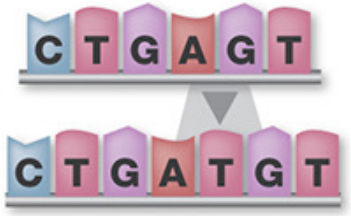
2. Choose the genetic variation (DNA) you have reason to believe might be involved



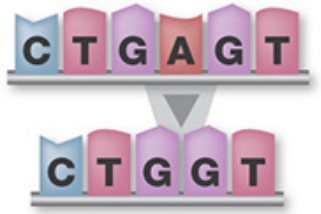
Point Mutation

AKA: Single nucleotide polymorphism (SNP)

AKA: Single nucleotide variant (SNV)

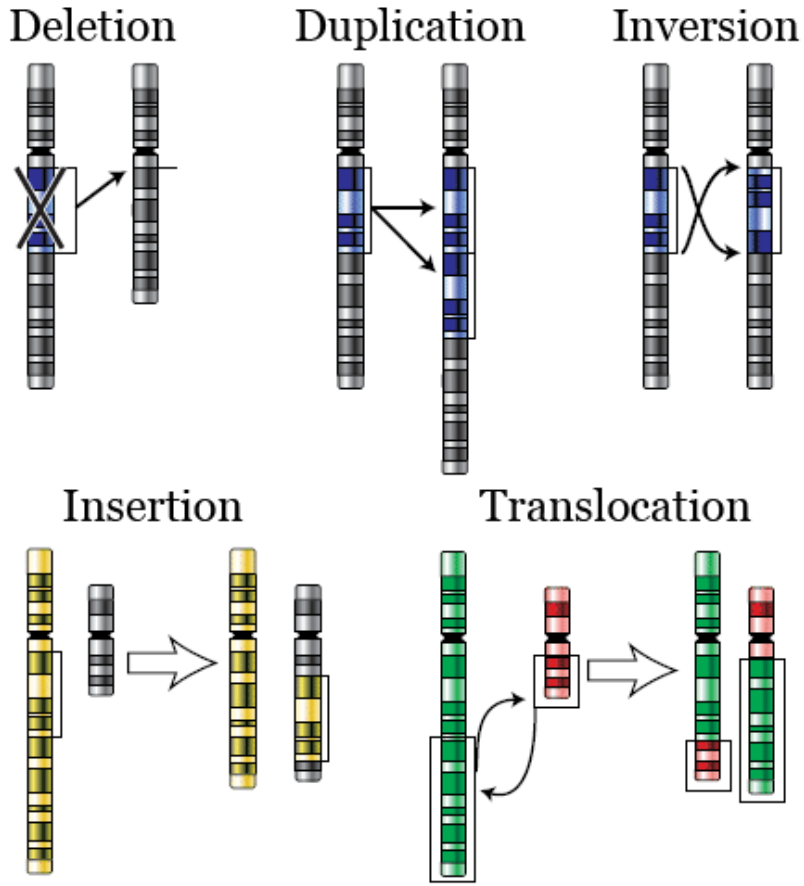


Insertion Mutation



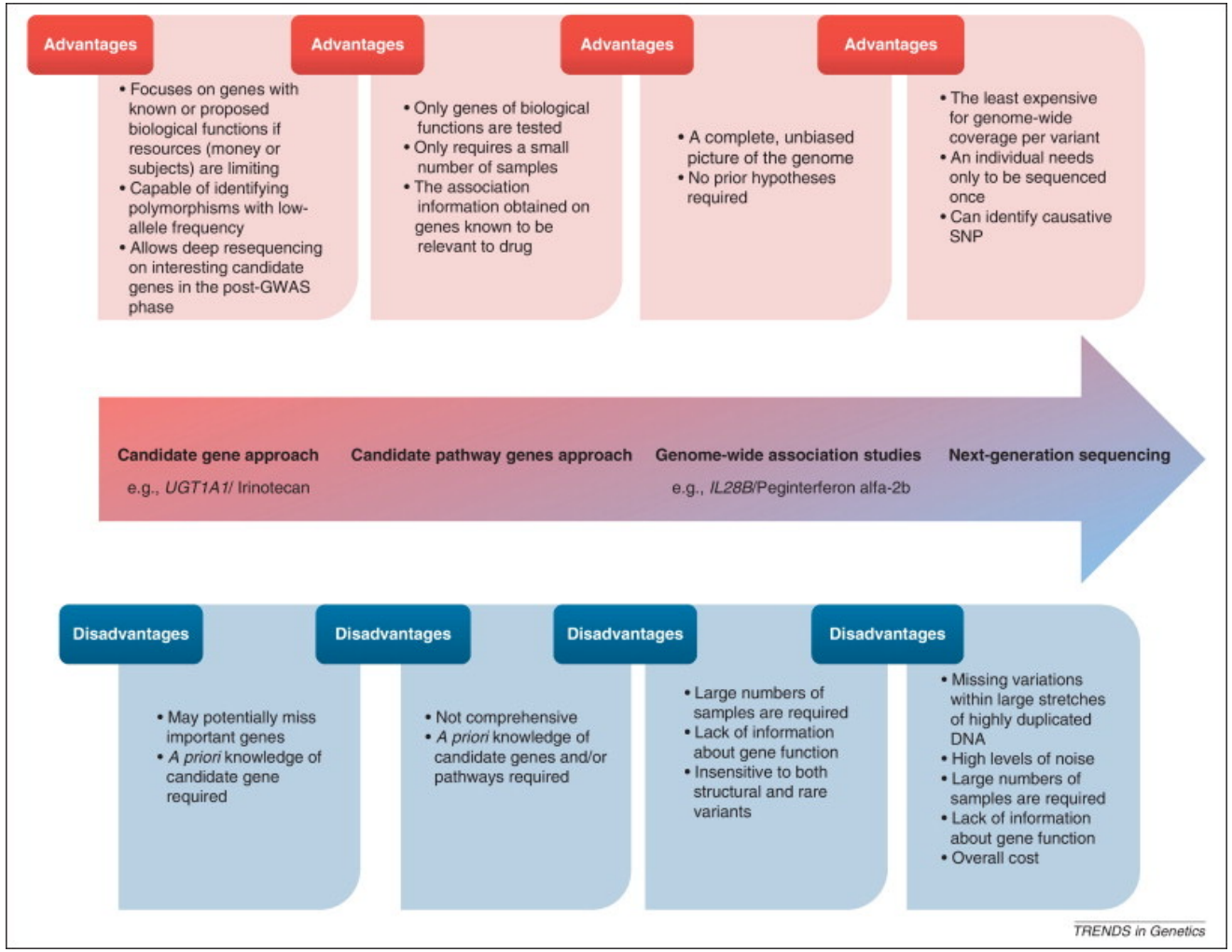
Deletion Mutation

https://learn.genetics.utah.edu/content/genetics/mutate/



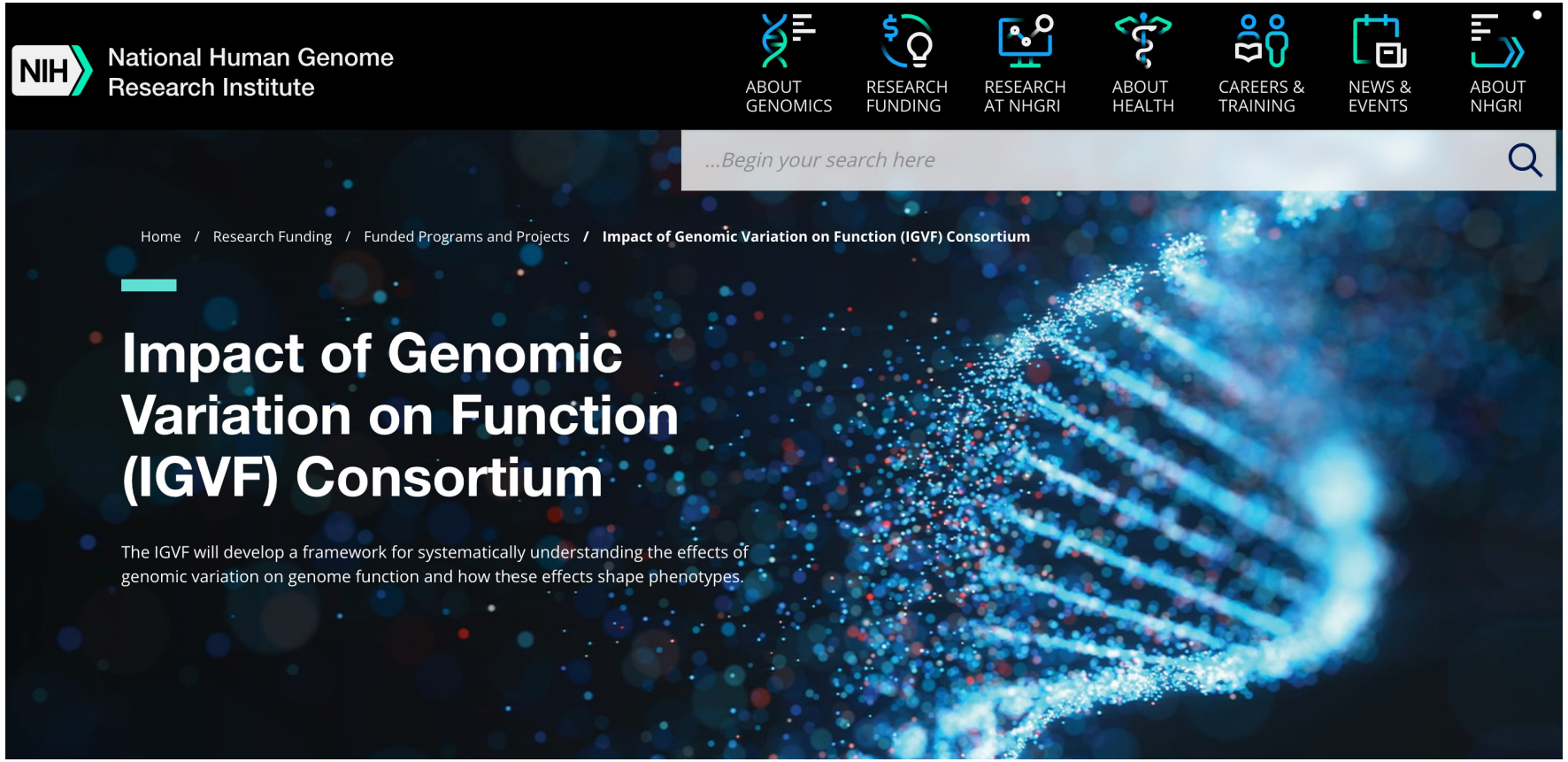
LibreTextsBiology

Strategies for identifying genetic variation that may be relevant to disease phenotype



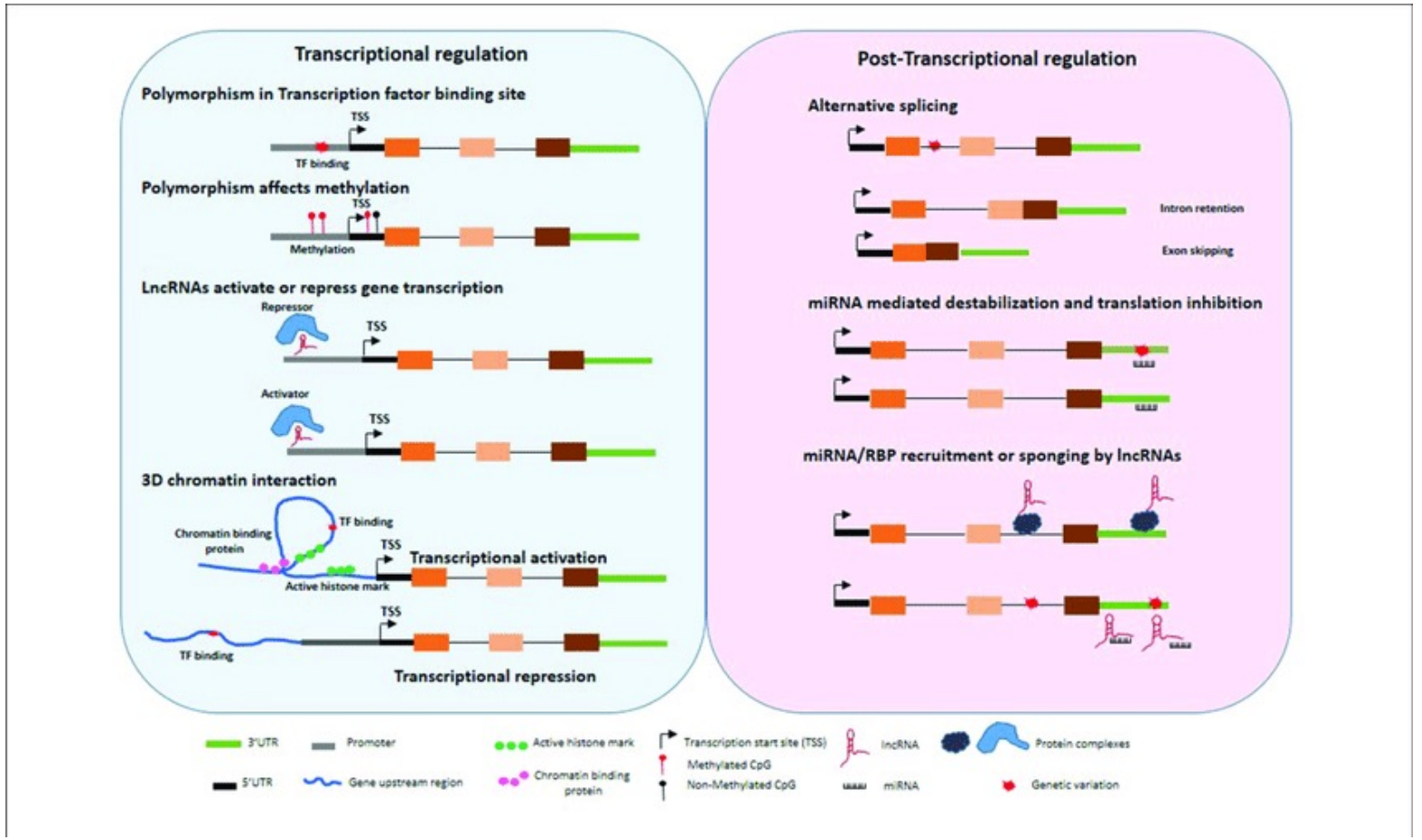
Madian et al., 2012, *Trend in Genetics*

Relating genetic variation to ”function” is one of the biggest and most active fields in current genetics research



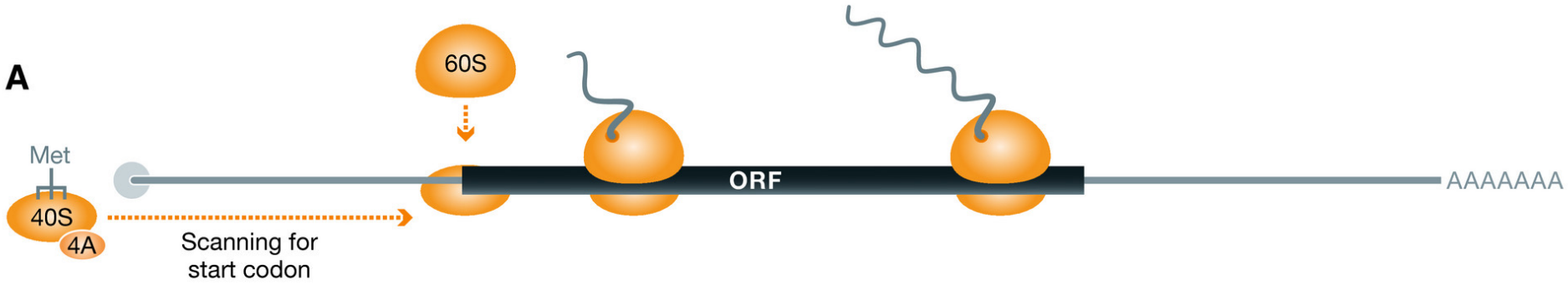
https://www.genome.gov/Funded-Programs-Projects/Impact-of-Genomic-Variation-on-Function-Consortium

3. Consider how your favorite variant may impact transcription (gene expression)



Ramsuran et al., 2018, *Frontiers in Immunology*

upstreamORFs (uORFs) and translation initiation

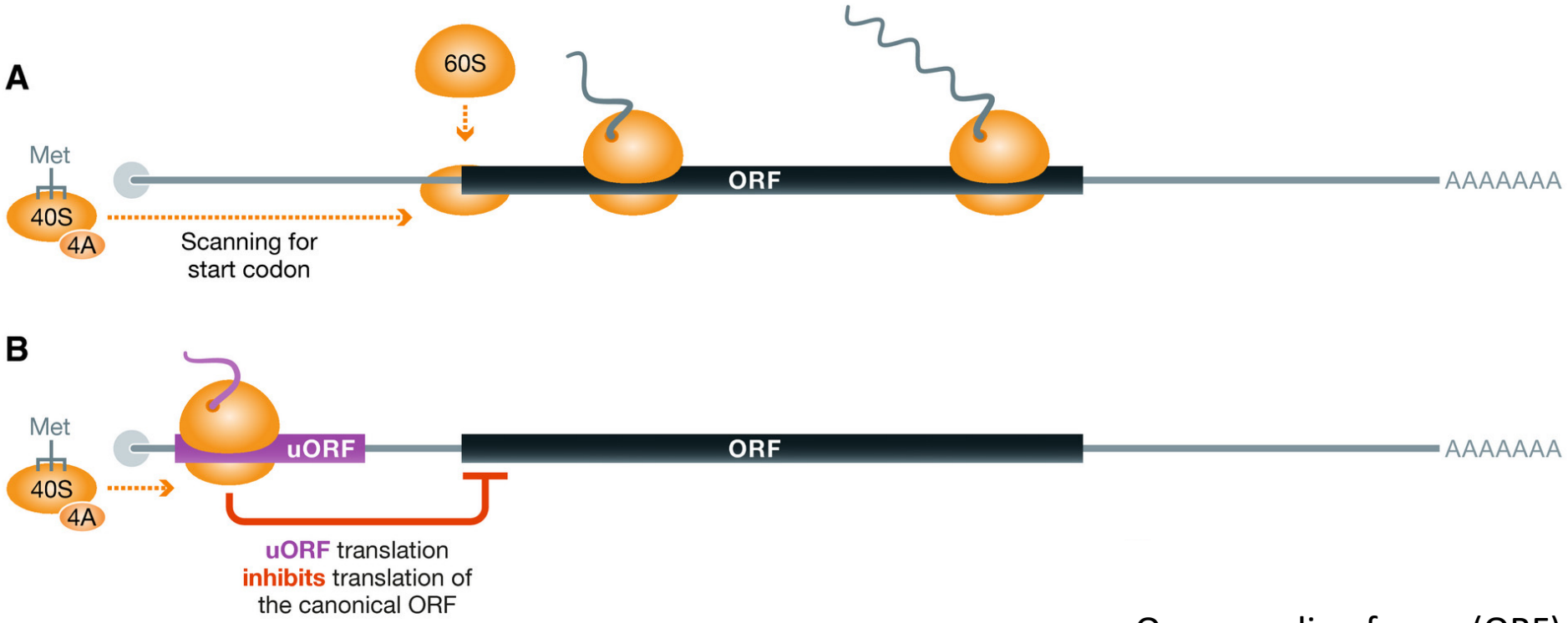


Open reading frame (ORF):

Any genomic region that has the correct upstream sequence motifs to be recognized by **ribosomes** and translated

Dodbele and Wilusz, EMBO Journal, 2020

upstreamORFs (uORFs) and translation initiation



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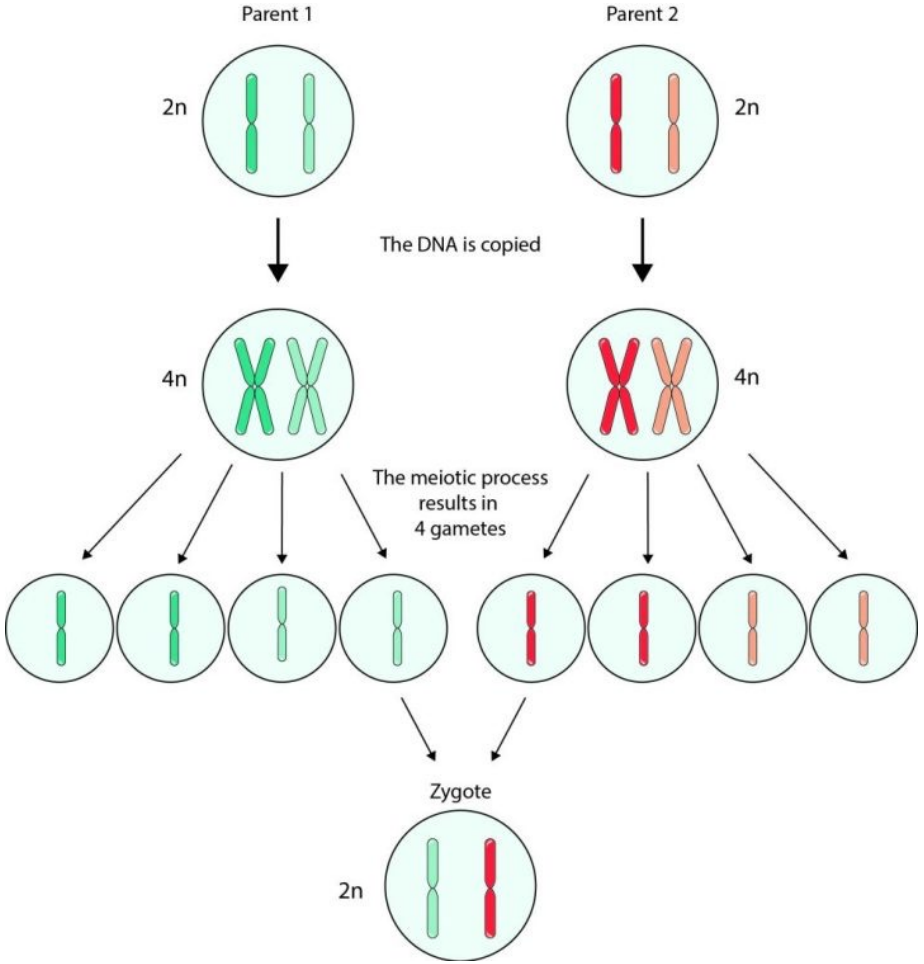
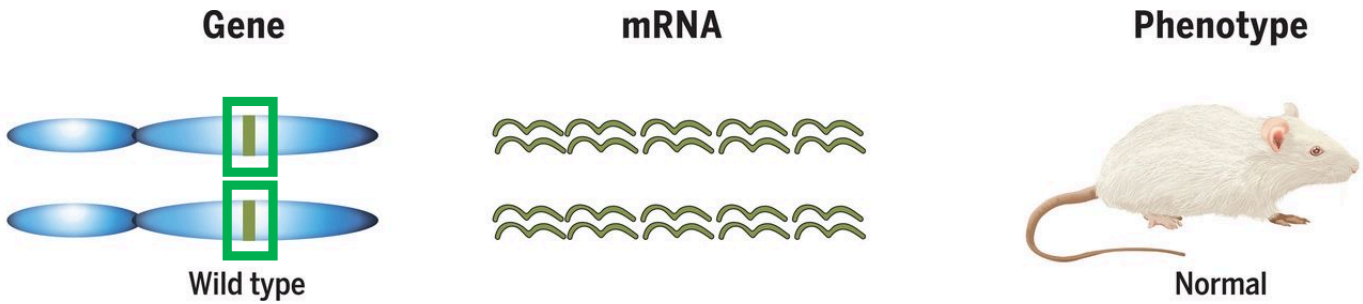
Any genomic region that has the correct upstream sequence motifs to be recognized by **ribosomes** and translated

Dodbele and Wilusz, EMBO Journal, 2020

What is haploinsufficiency and how does it impact transcription?



sitn.hms.harvard.edu



Matharu et al., 2018, *Science*

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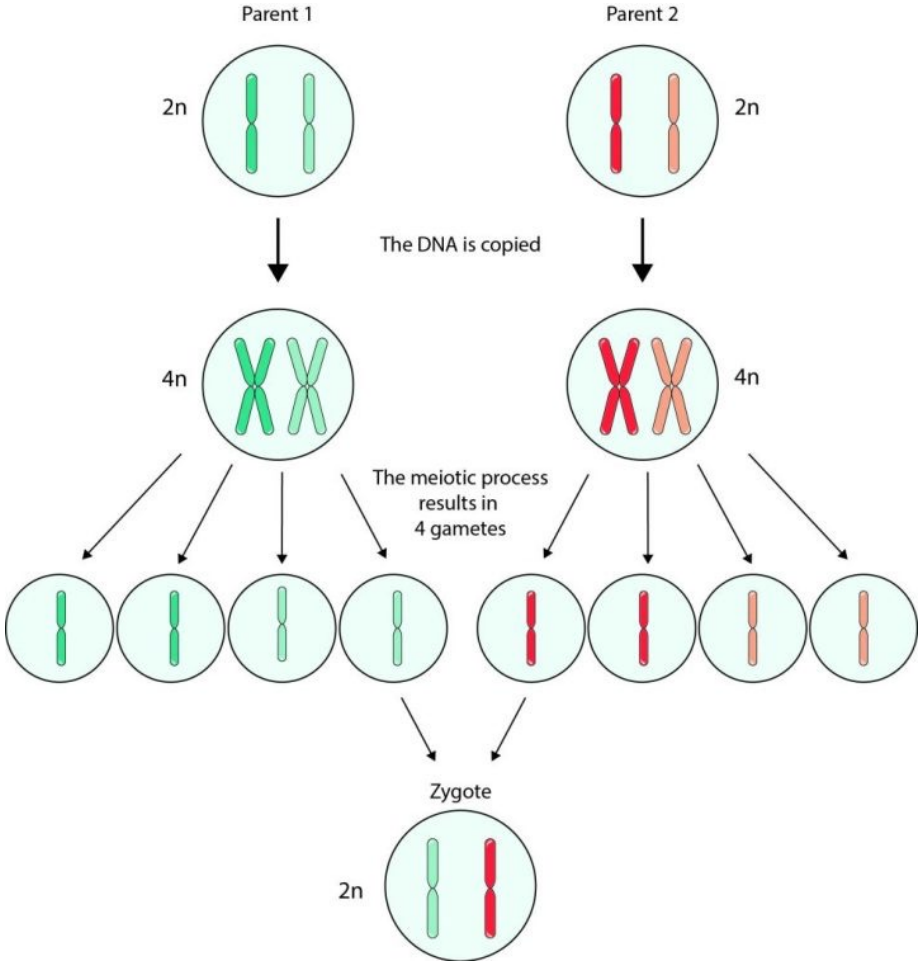

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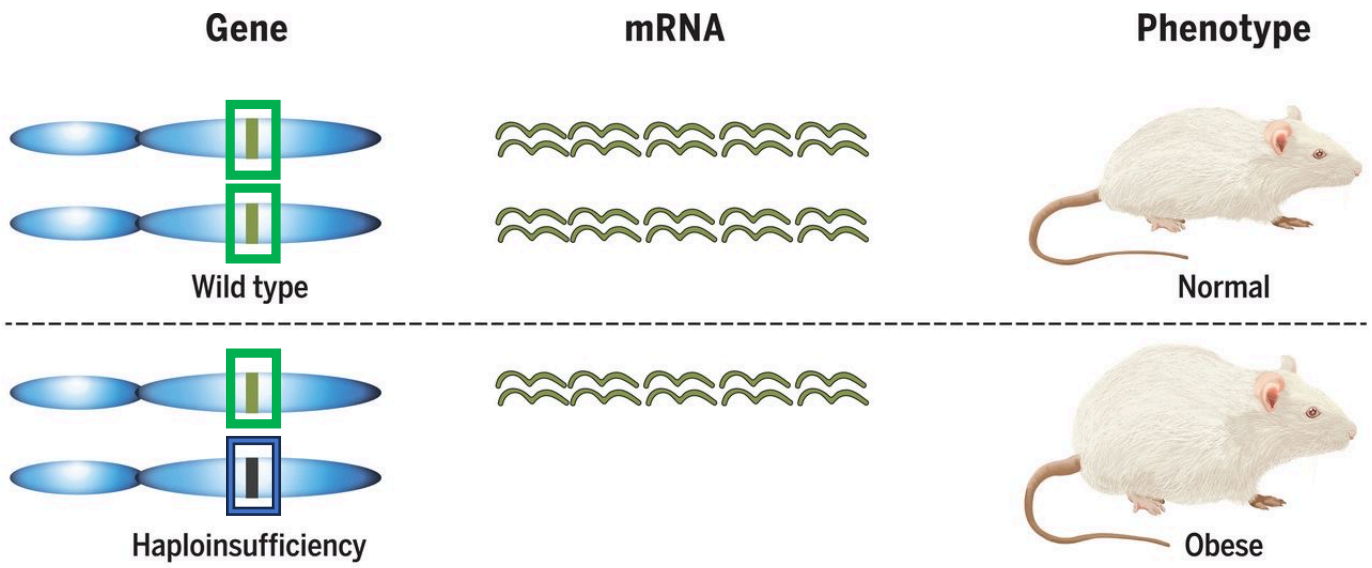

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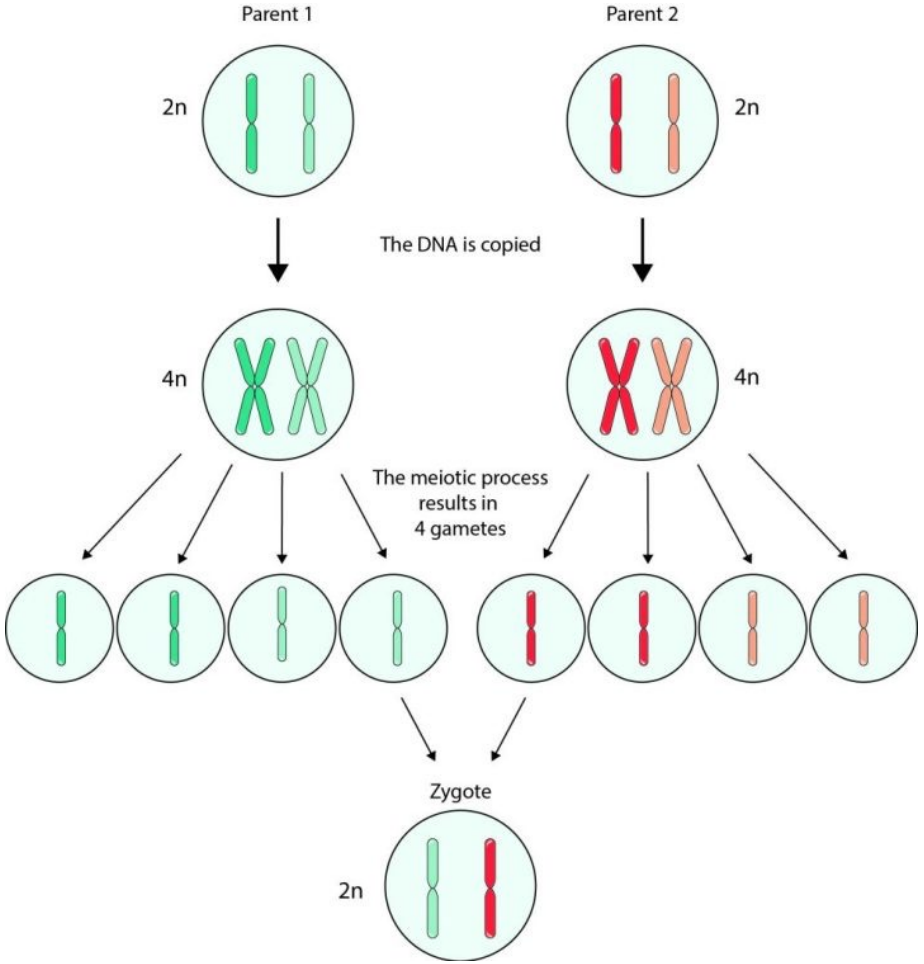

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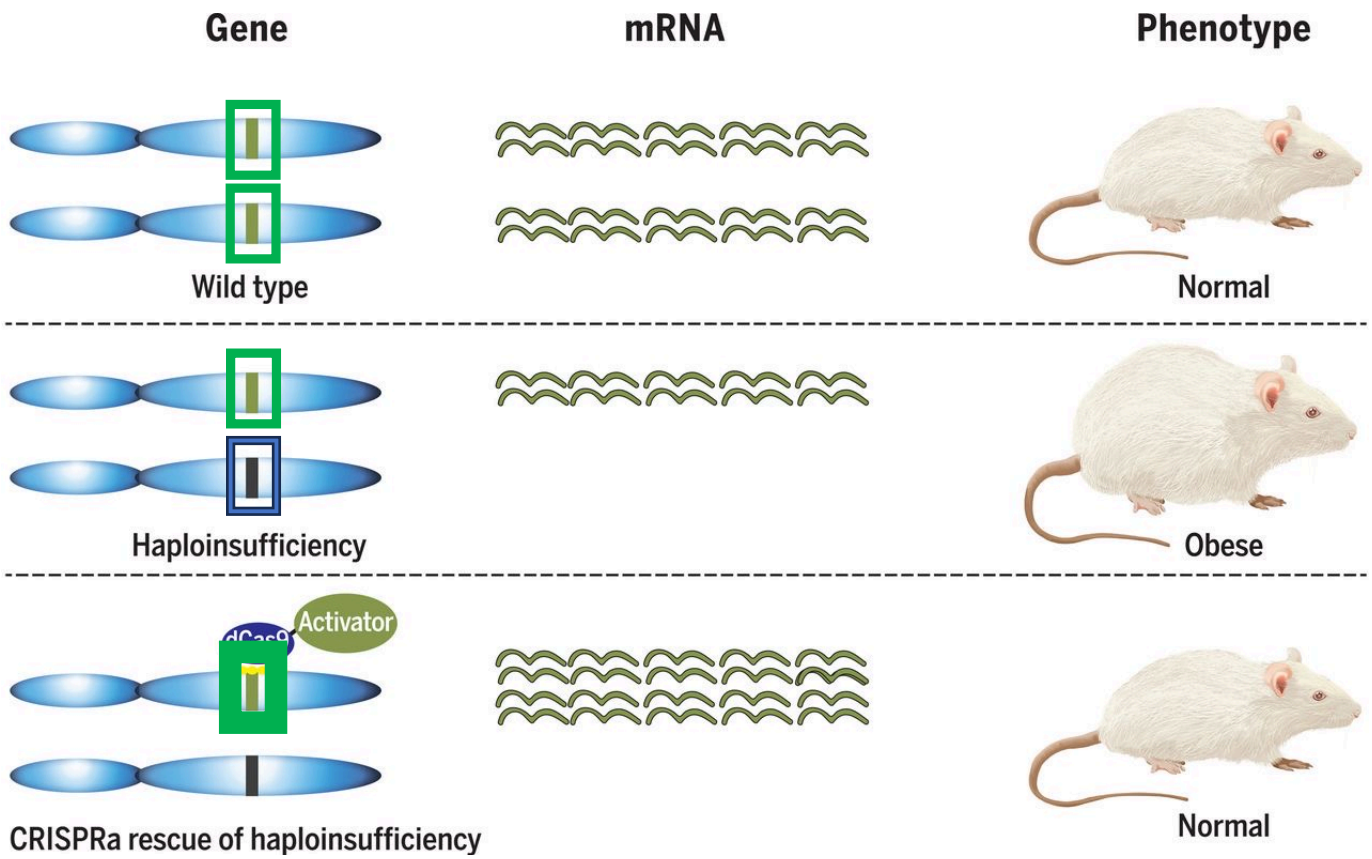
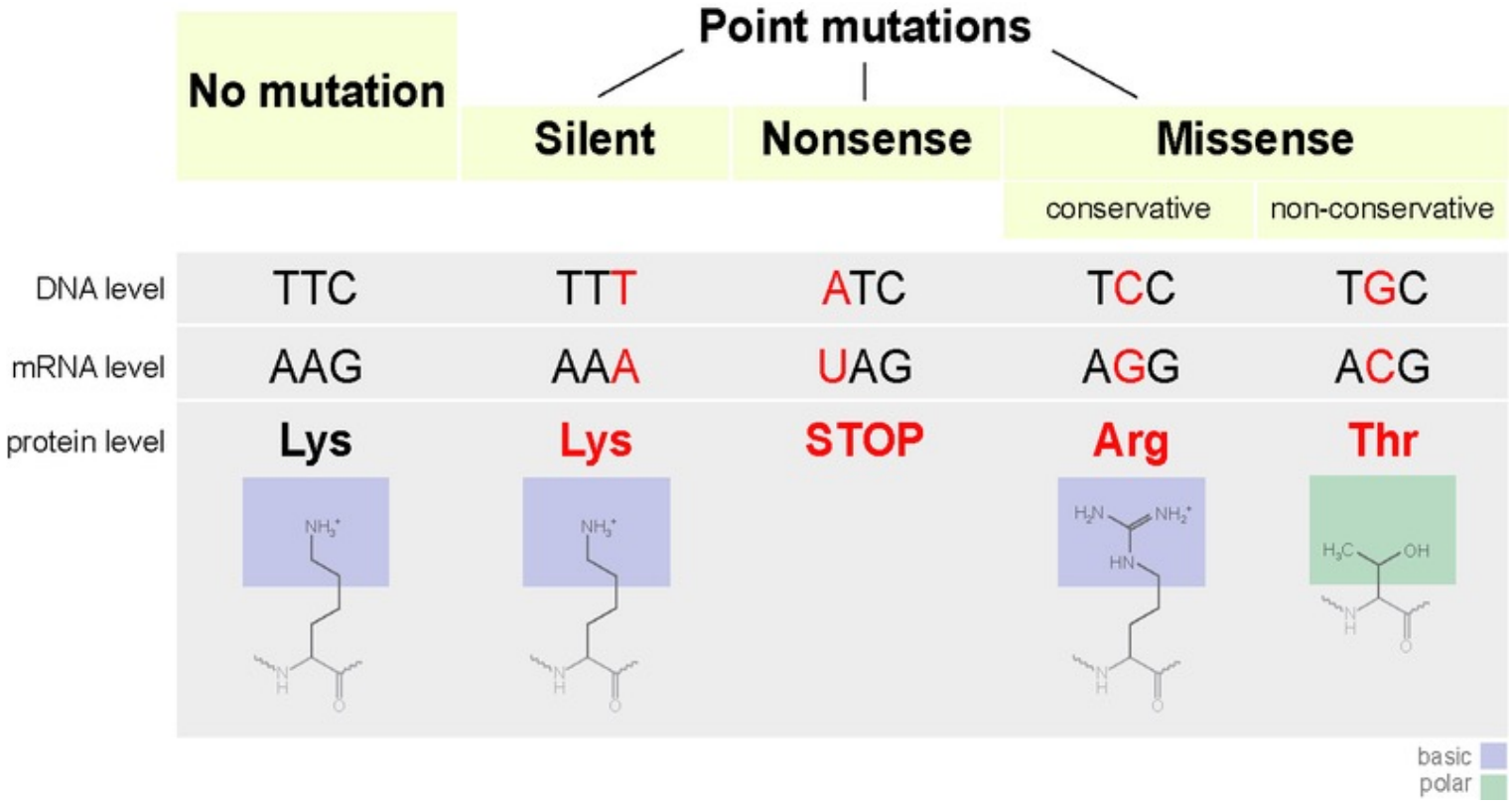


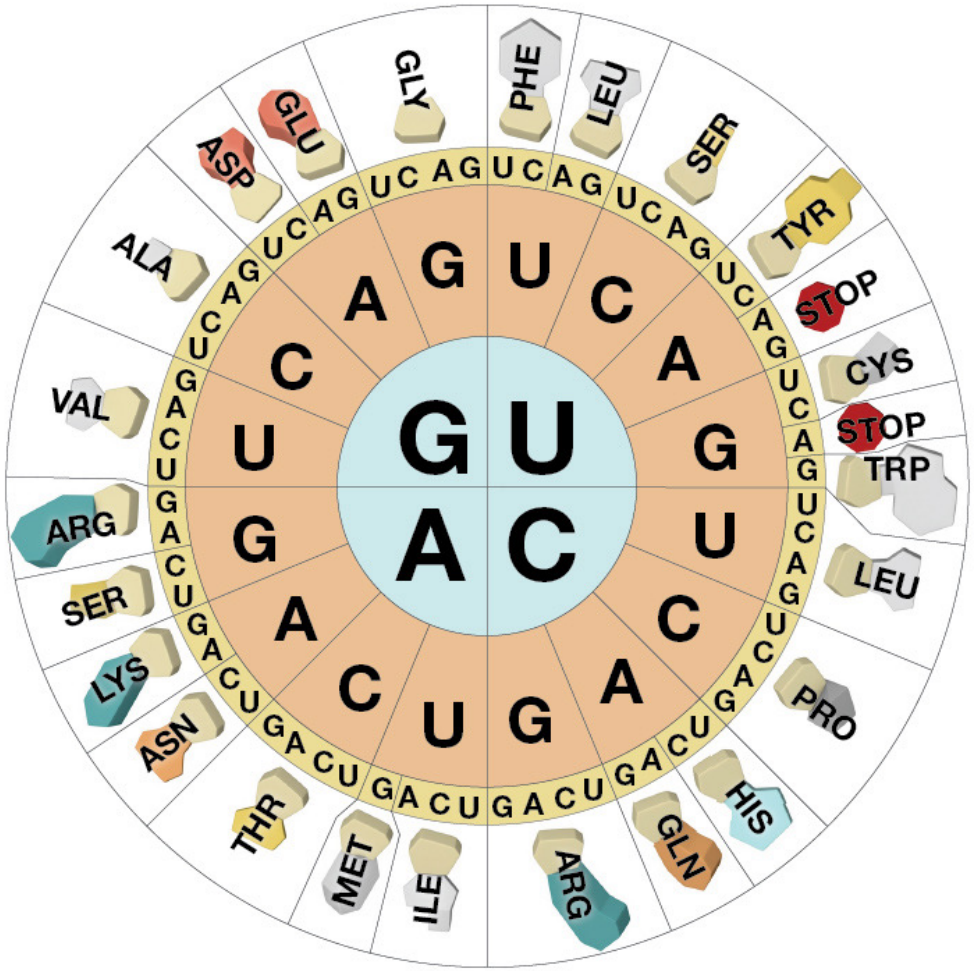
Matharu et al., 2018, *Science*

4. Consider how your favorite variant may impact translation (protein expression)



https://www.ebi.ac.uk/training/online/courses/human-genetic-variation-introduction/what-is-genetic-variation/what-effect-do-variants-in-coding-regions-have/

Protein structure == protein function



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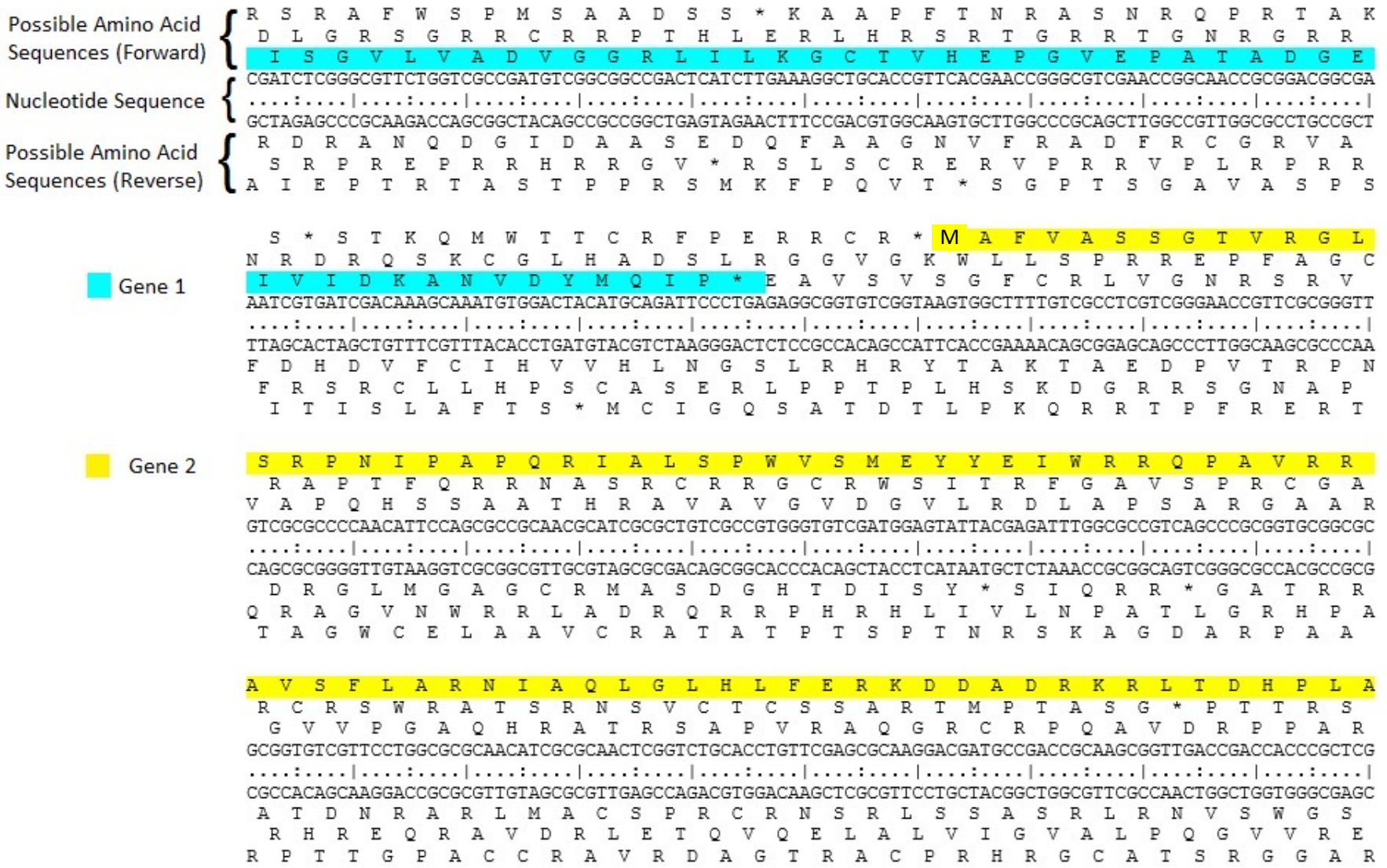
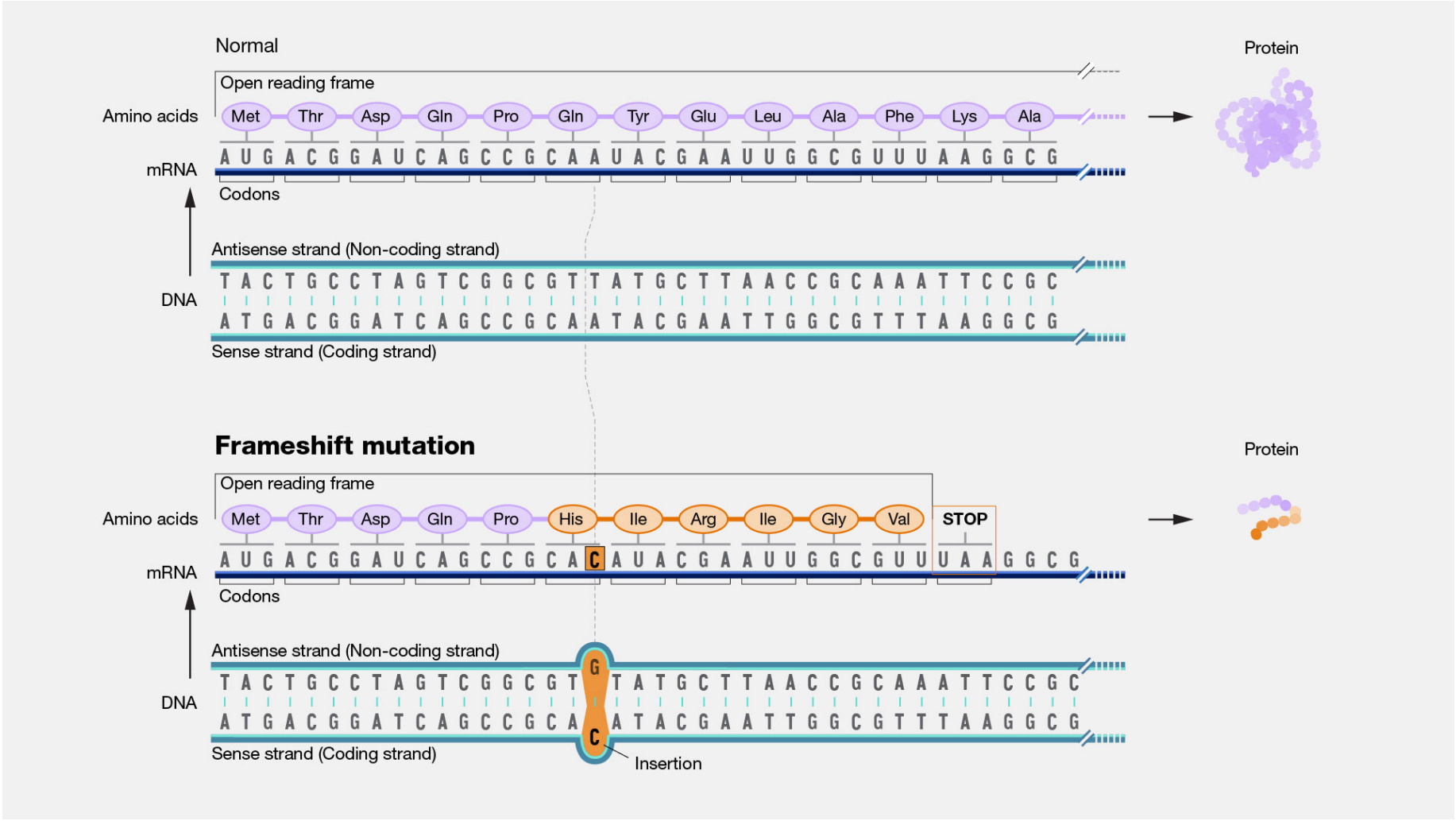
Frameshift mutations can change the coding sequence (CDS) that gets translated



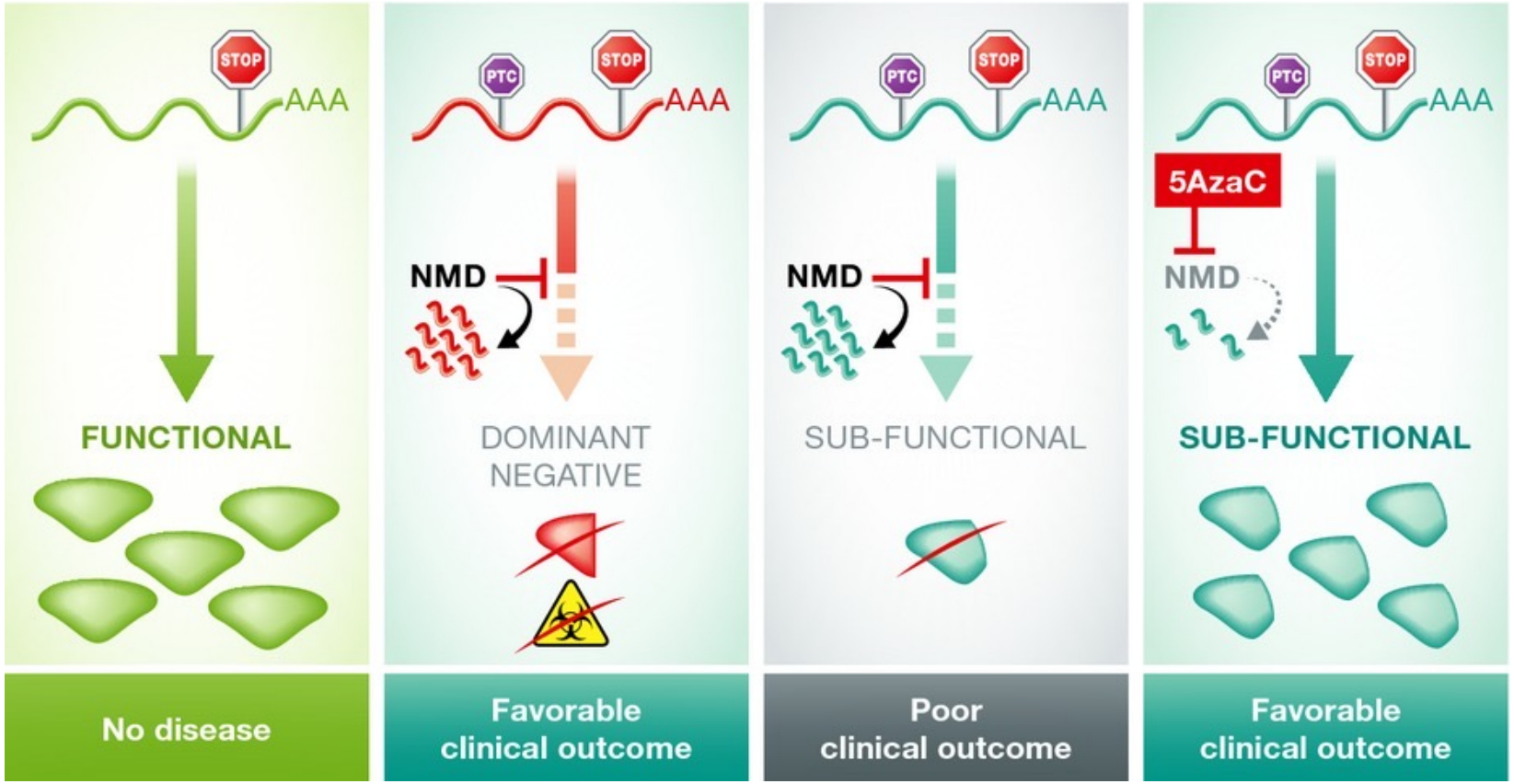
Wikipedia

Frameshift mutations can change the coding sequence (CDS) that gets translated



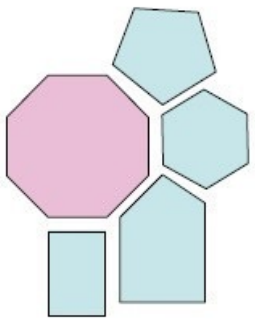
https://www.genome.gov/genetics-glossary/Frameshift-Mutation

5. Come up with a hypothesis for how you think your favorite variant may be impacting your disease/phenotype of interest



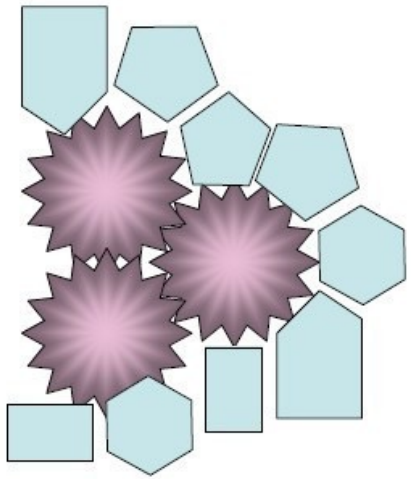
Shao and Wilkinson, 2014, *EMBO Mol Med*

Wild-type



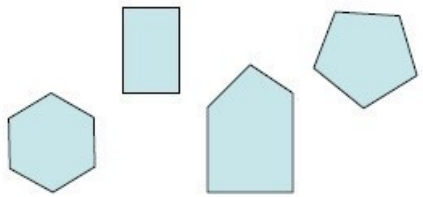
- Complex formed
- Normal cell physiology

Gain-of-function



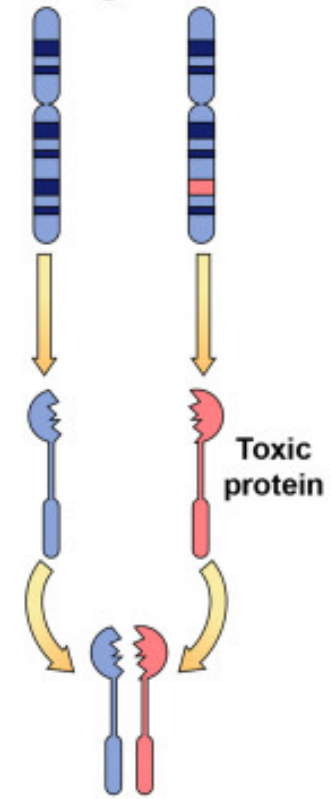
- Hyperactivation of signalling pathways
- Formation of protein aggregates

Loss-of-function



- Complex not formed
- Complex function not performed

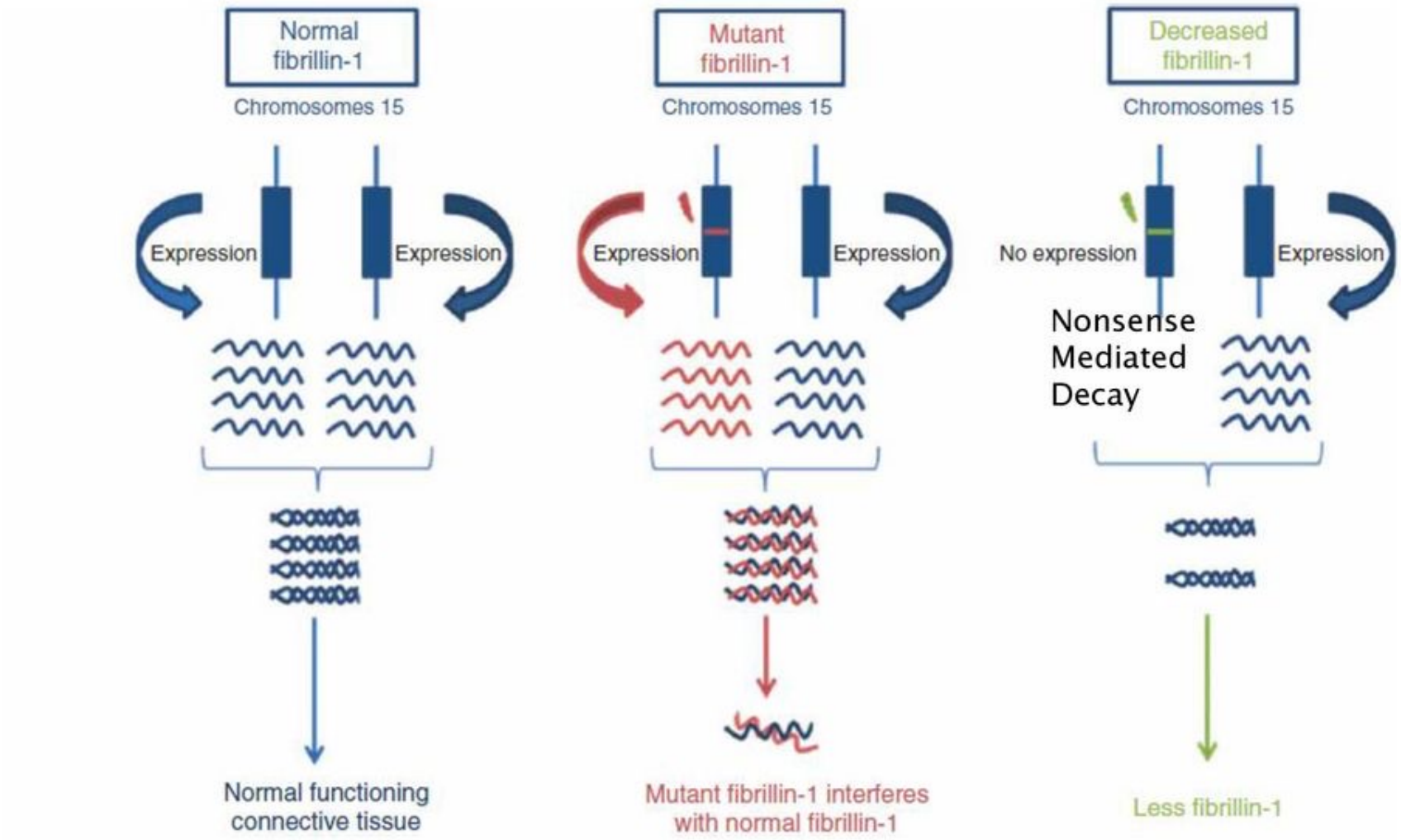
Dominant Negative



Segalat, 2007, *Orph. J. Rare Dis.*

Gonzaga-Jauregui et al., 2021, *Gen Rare Dis.*

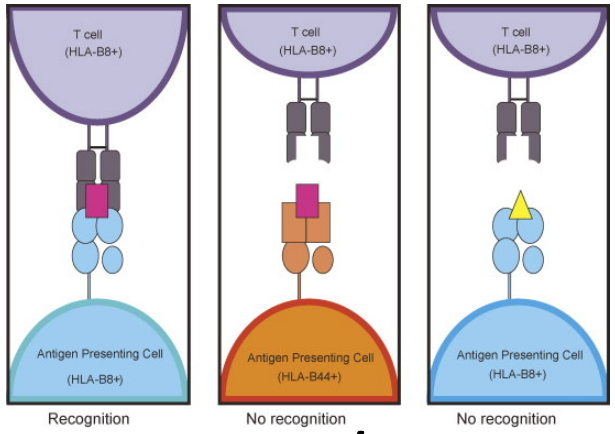
dominant negative vs haploinsufficiency



Franken R, Expert Opin Orphan Drugs 2014 Aug;2(10):1-14.

6. Design *in silico/in vitro/in vivo* experiments to test

The signatures of function are passed on through our DNA



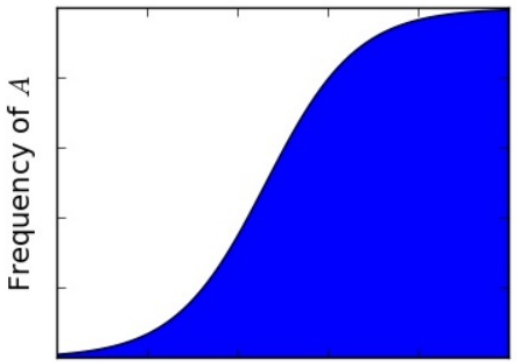
Function/trait

Sequence

Fitness

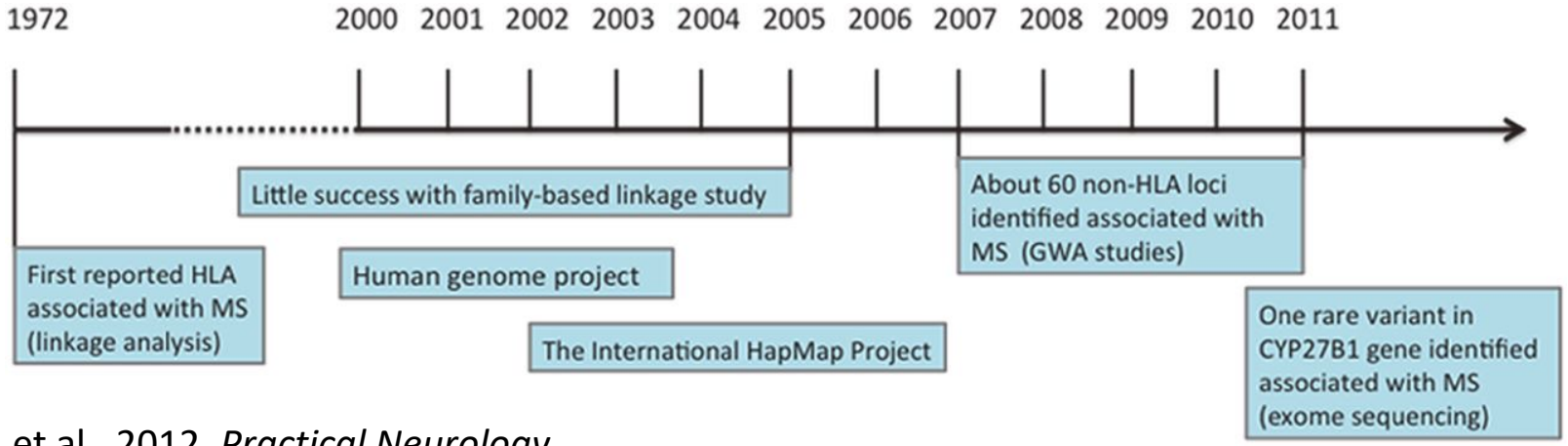
H. sapiens
S. cerevisiae
M. tuberculosis
E. coli

Protein amino acid sequence



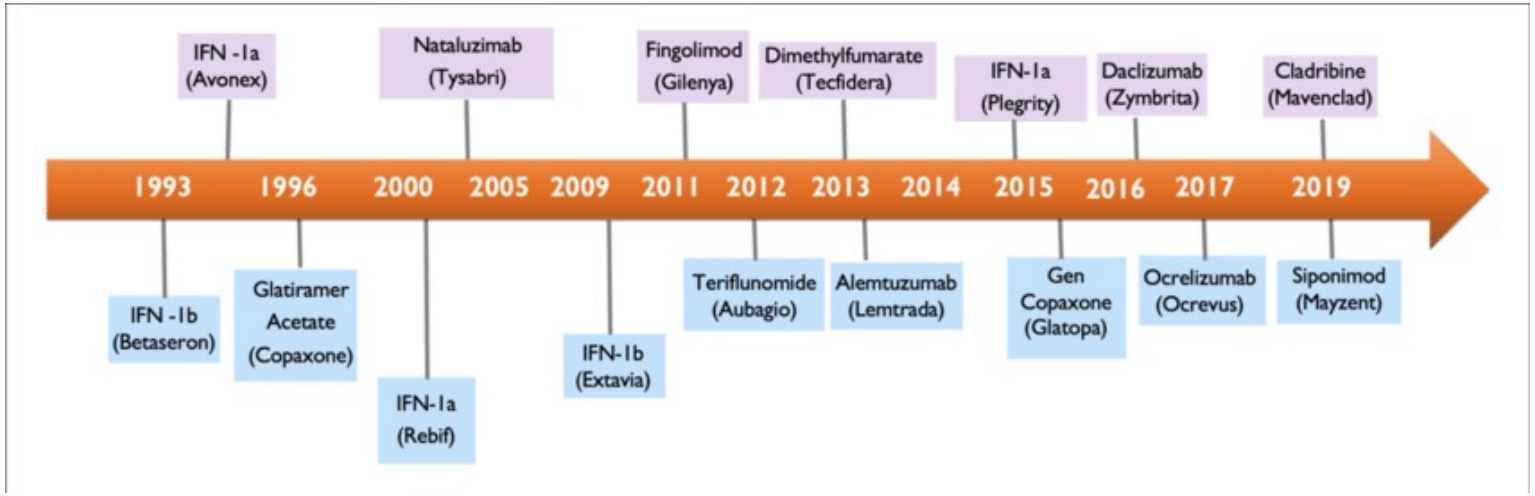
Why are the mechanisms we build from our understanding of basic biology so important for how we think about disease?

Multiple sclerosis has been considered a disease of the immune system since the 1970s



Lin et al., 2012, *Practical Neurology*

Our interpretation of disease mechanism shapes clinical treatment



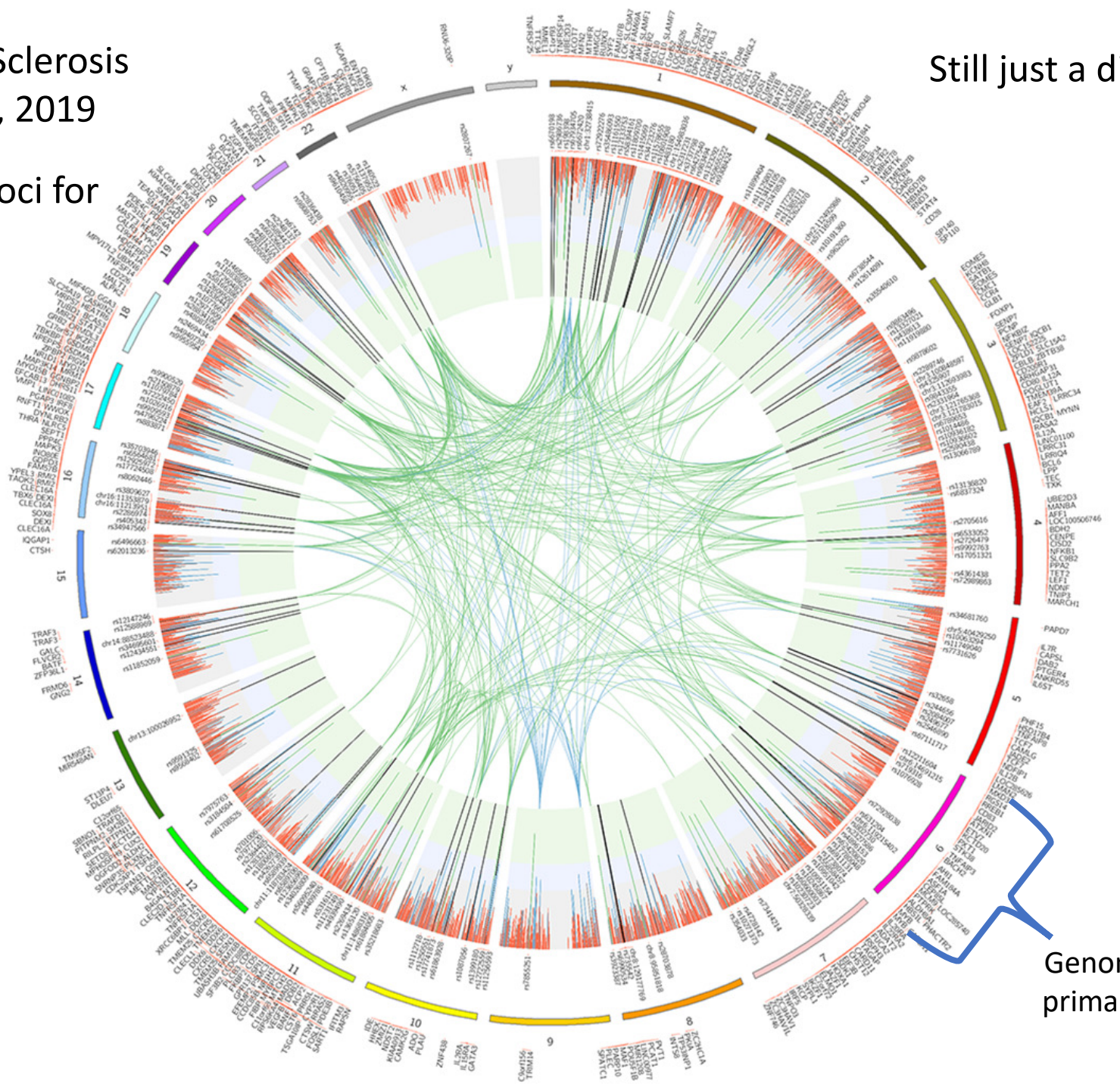
Melamed and Lee, 2020, *Front. Imm*.

"Various clinical and experimental findings suggest a pathogenic role of antibodies in multiple sclerosis (MS). **Yet, whether antibodies contribute to the pathogenesis or progression of MS is still a subject of intense debate."**

- den Dunnen, et al., 2021, *Neural Regen Res.*

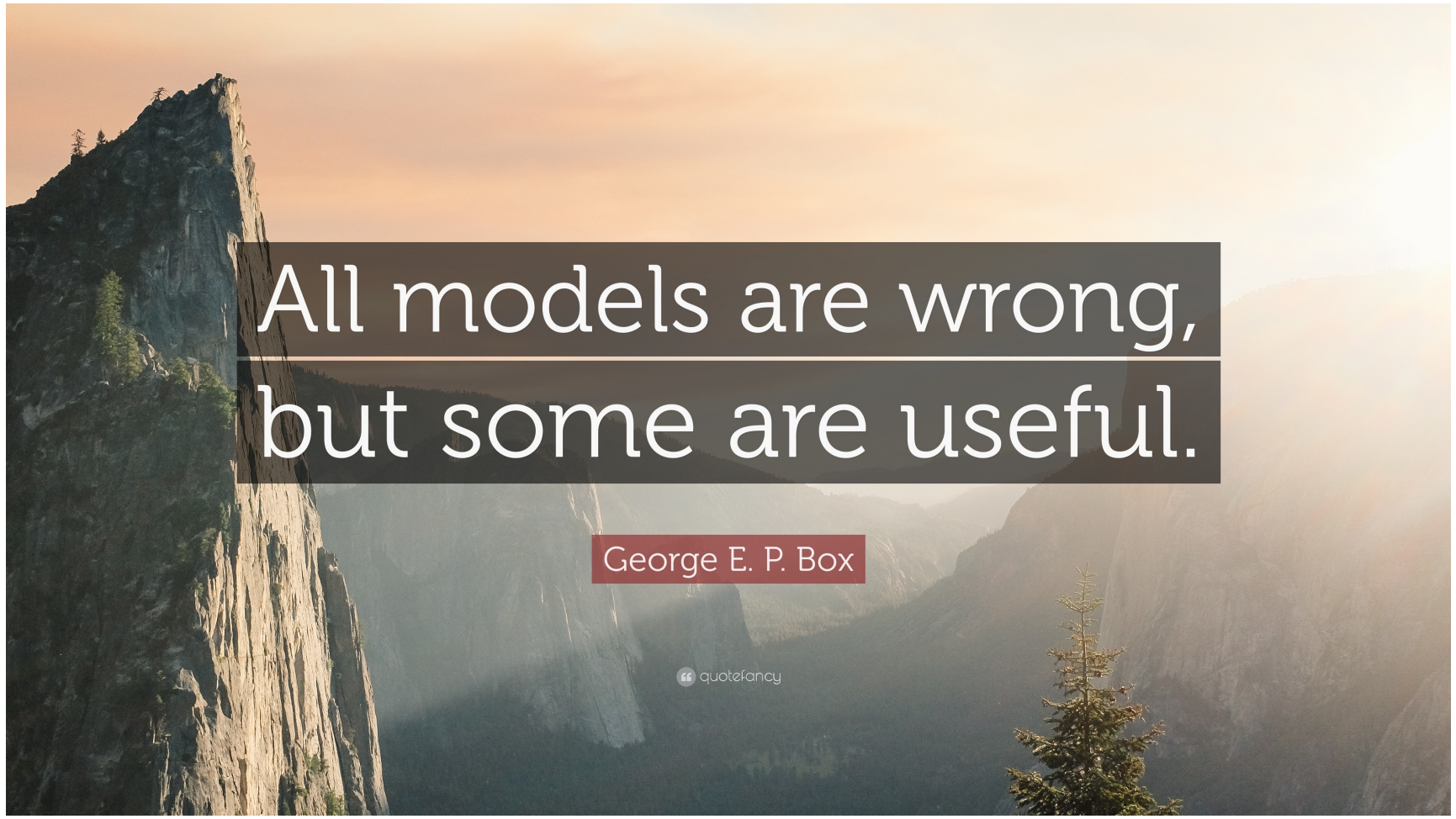
International Multiple Sclerosis Genetics Consortium, 2019

>200 genome wide risk loci for multiple sclerosis



Still just a disease of the immune system?

Genomic region containing primary immune risk genes



We are all just figuring this out as we go, based on what is known at any given moment… which is constantly changing as we learn more.

So state your assumptions and don’t take things too seriously.

Thanks for your interest!

- Feel free to email me: kristen.wade@ucsf.edu