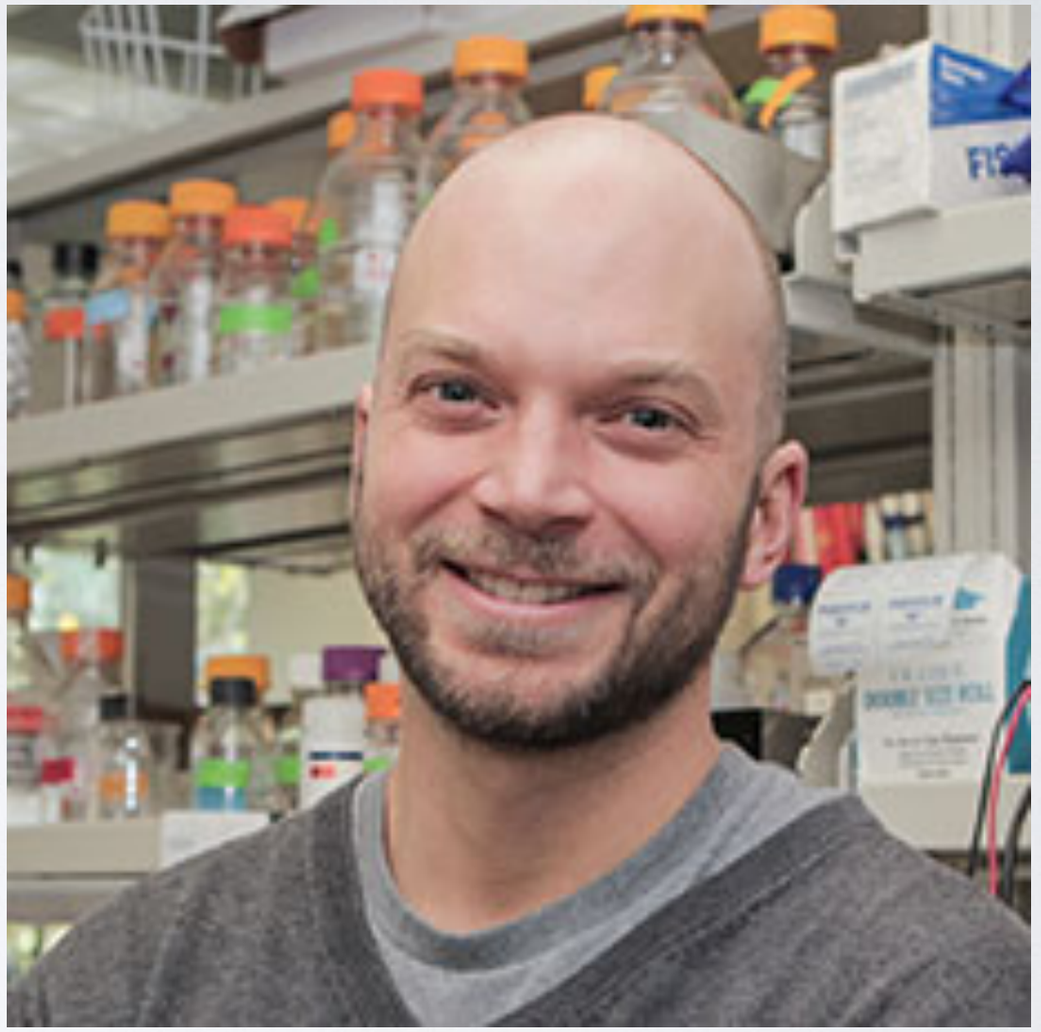
Lecture 3- Vignette 1

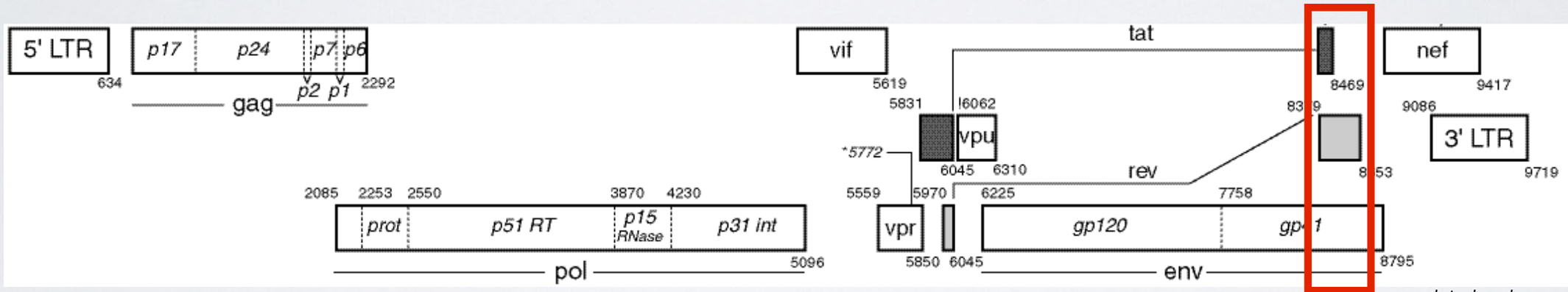
Discovery of a novel overprinting gene in polyomaviruses



Matt Daugherty, former postdoc
now Assistant Professor, UC San Diego

What is overprinting? How does it evolve?

DNA	**CCTATTAATGGAACAGCCA**
Frame 1	**P I N G T A**
Frame 2	**L Y M E Q P**



hiv.lanl.gov

UNUSUAL EVOLUTIONARY CONSERVATION

Virus A	**CCTATTAATGGAACAGCCA**	
Frame 1	**P I N G T A**	
Frame 2	**L L M E Q P**	
Virus B	**CCTAT<u>A</u>AATGGAACAGCCA**	
Frame 1	**P I N G T A**	***Synonymous change***
Frame 2	**L * M E Q P**	***Nonsynonymous change***

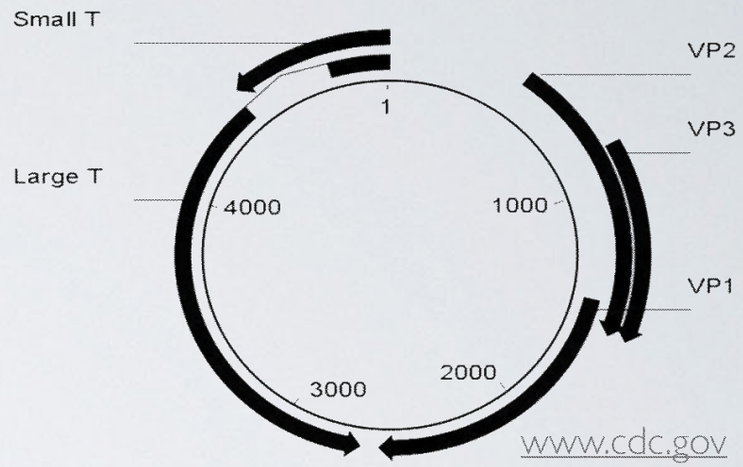
Observe depression of 'synonymous' mutations in overprinting regions

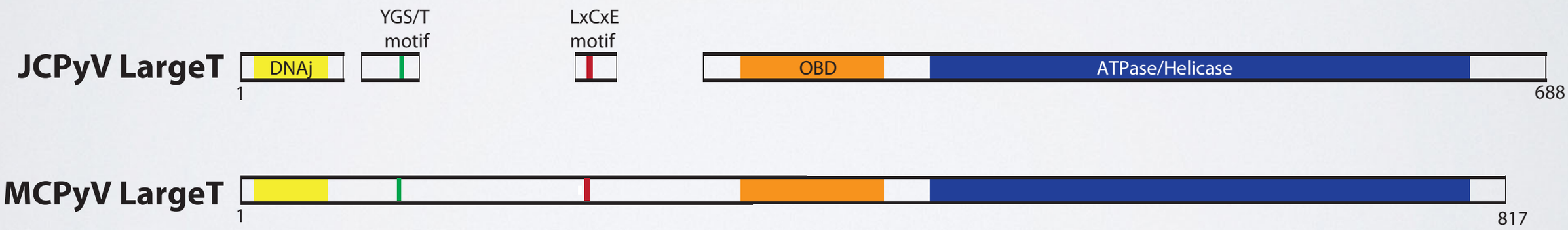
A NOVEL GENE IN MCPYV

Polyomaviruses (PyV's) are small DNA viruses

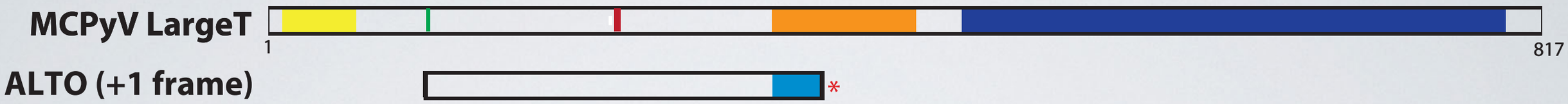
9 distinct human viruses, infect >50% of people

SV40: Causes cancer in monkeys (also has a very useful promoter)
MousePyV: Causes cancer in rodents
Merkel cell polyomavirus (MCPyV): Causes cancer in humans

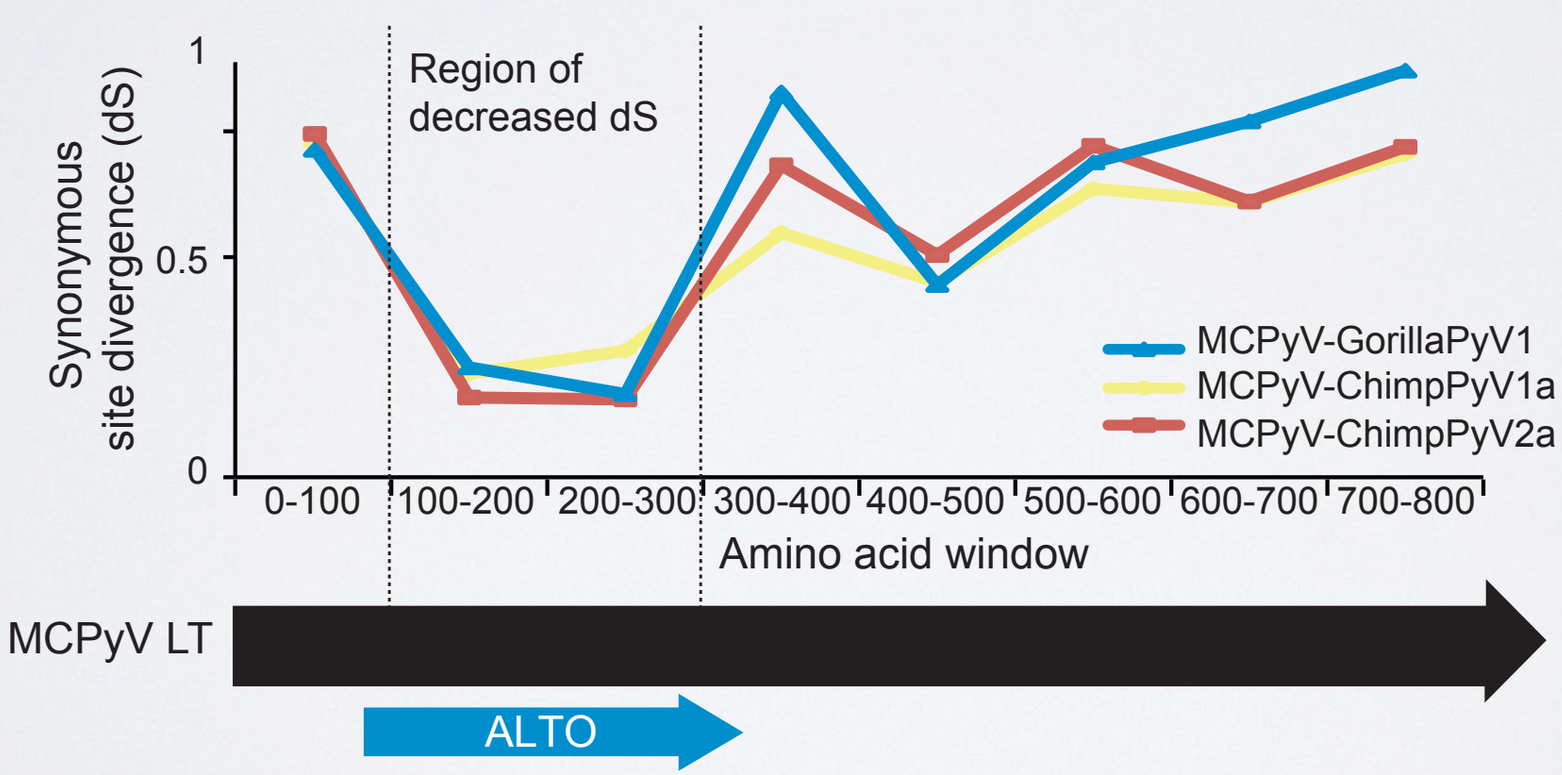




ALTERNATE T ANTIGEN ORF (ALTO)



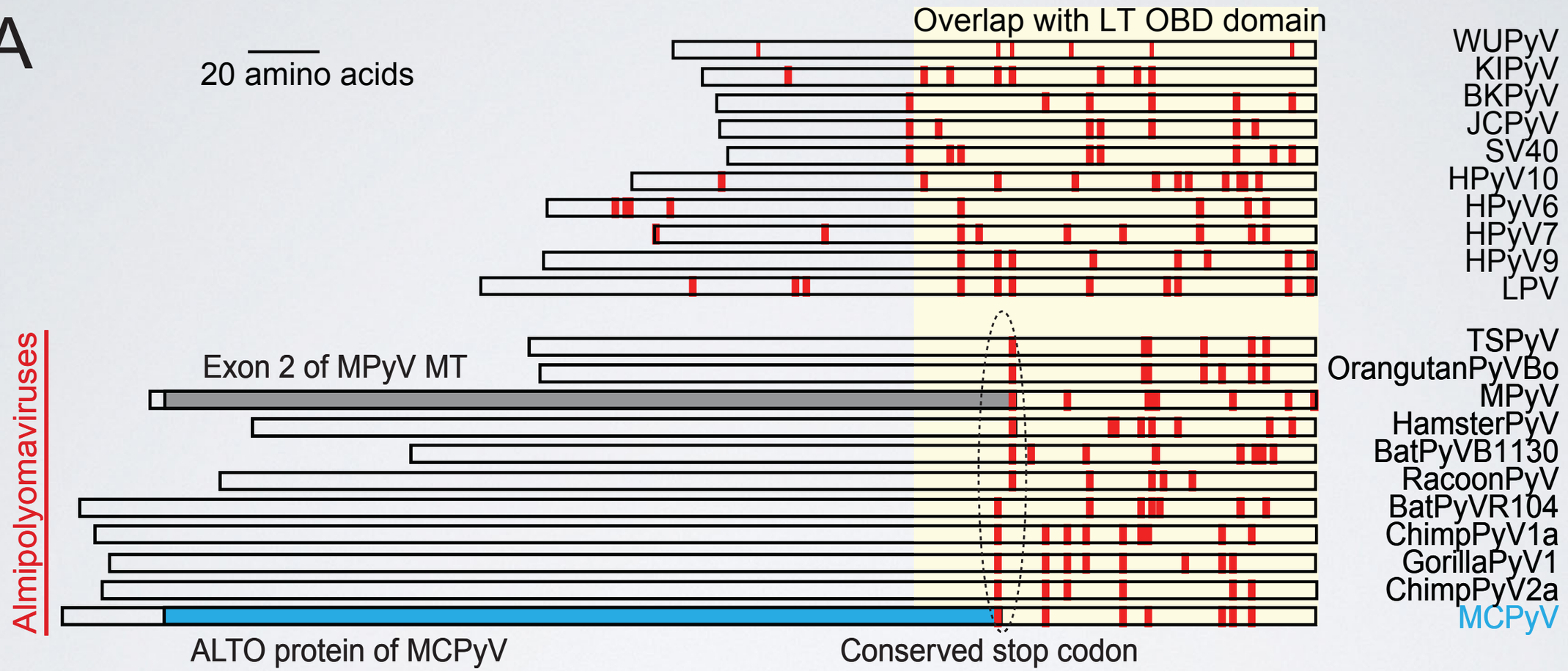
248 amino acid protein
No recognizable primary or secondary structure except a hydrophobic C-terminus
No similarity to any other viral protein except 3 closely related viruses



How did ALTO come to exist?

A WHOLE CLADE OF OVERPRINTED LT GENES

Are there other PyVs with a large uninterrupted ORF overprinting on LT?

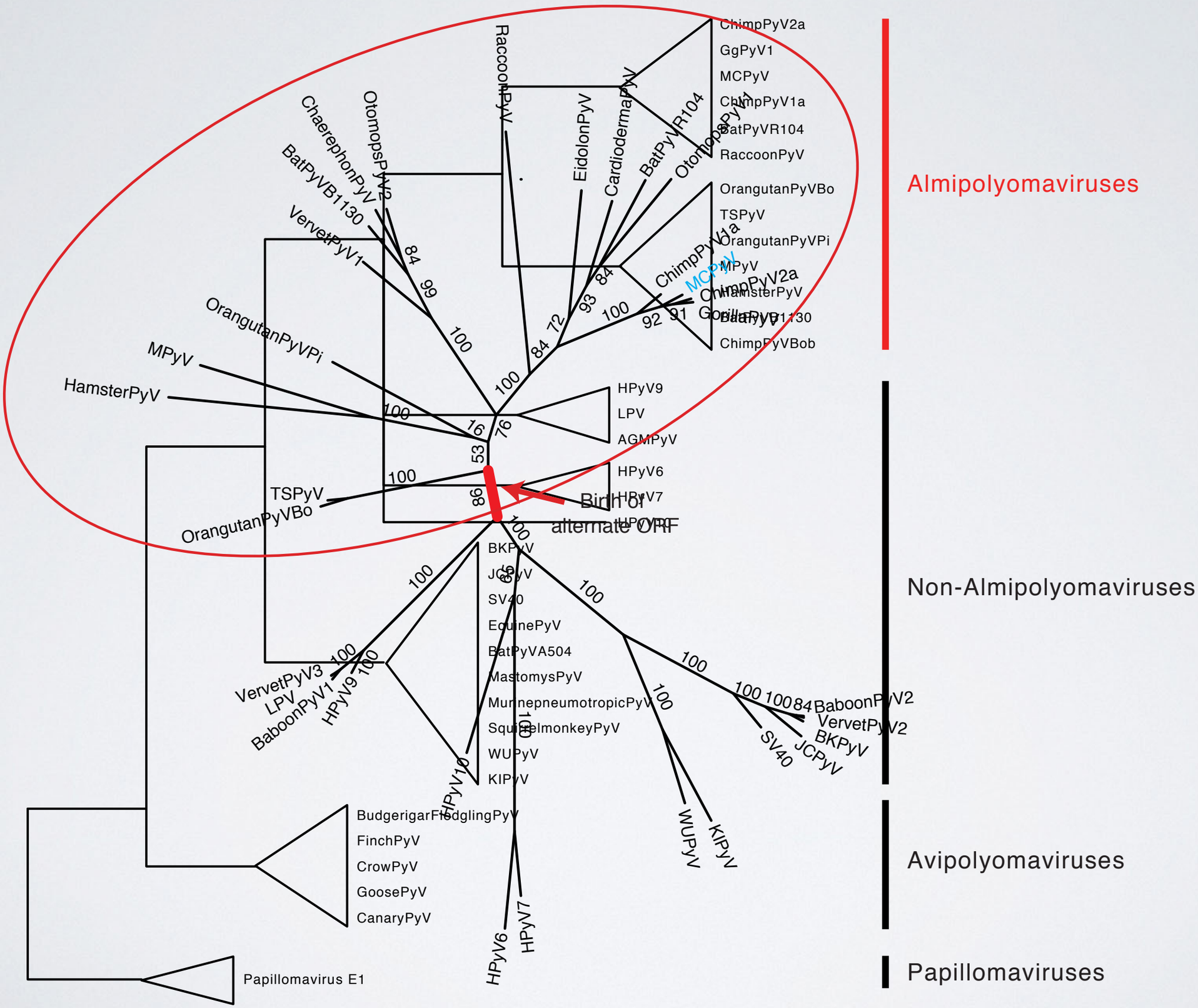


Includes another human virus (TSPyV) and oncogenic MousePyV

The overprinting MousePyV gene (called MiddleT) is sufficient to cause cancer

Defines a clade of Almi(ALTO or MiddleT containing)polyomaviruses

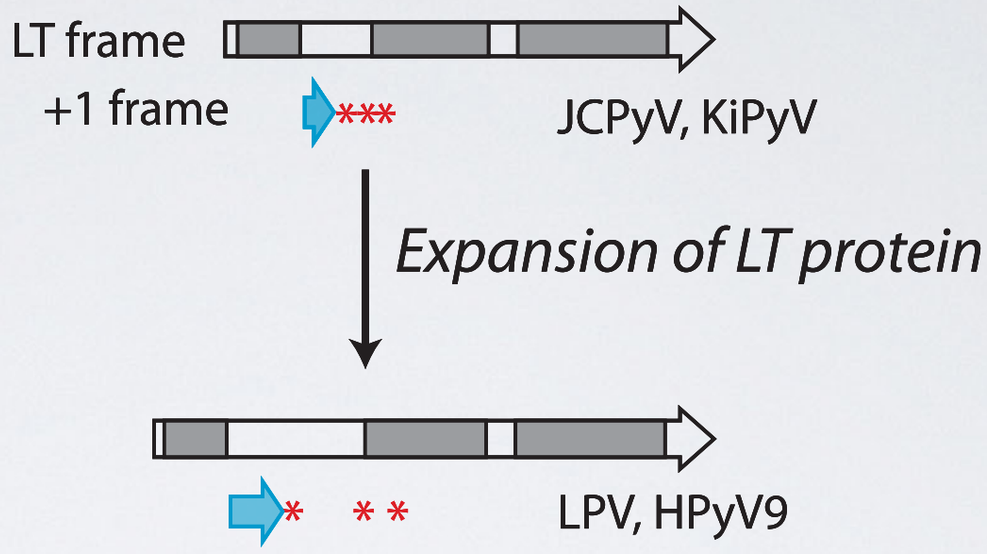
ALTO WAS BORN IN ONE VIRAL LINEAGE



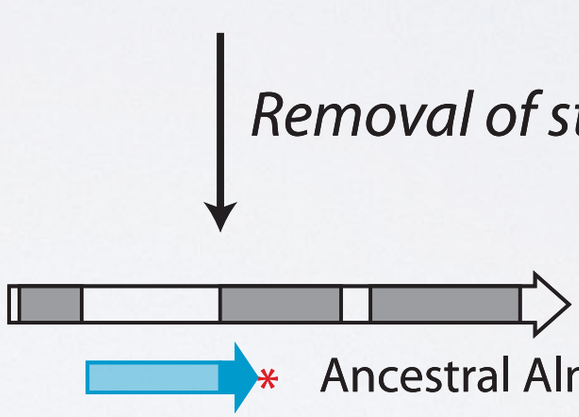
A STEPWISE MODEL FOR OVERPRINTING BIRTH

Ancestral LT gene

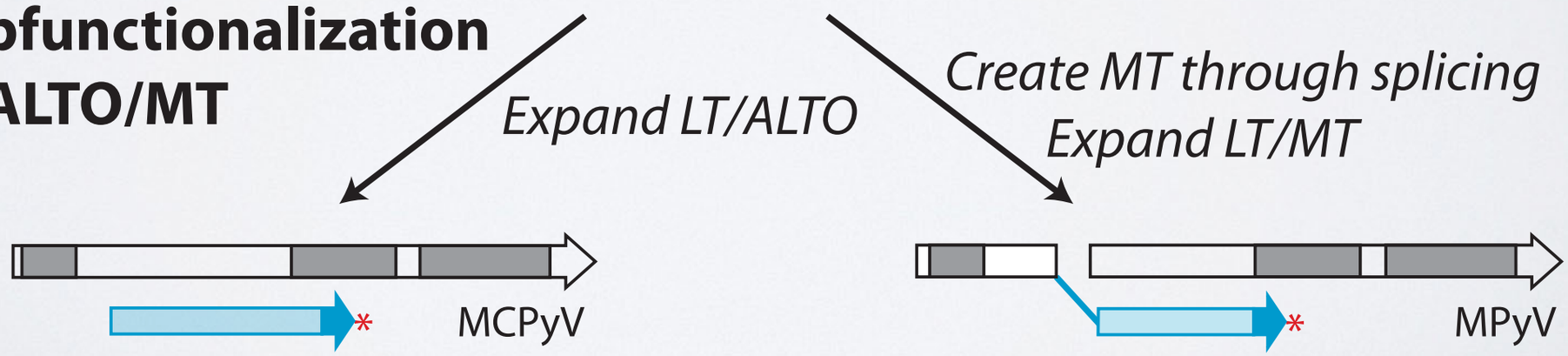
Potential for +1 frame start codon and hydrophobic motif already present



Innovation of ALTO/MT

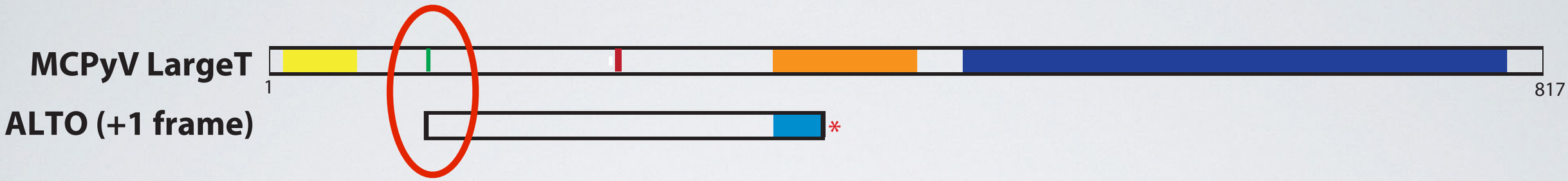


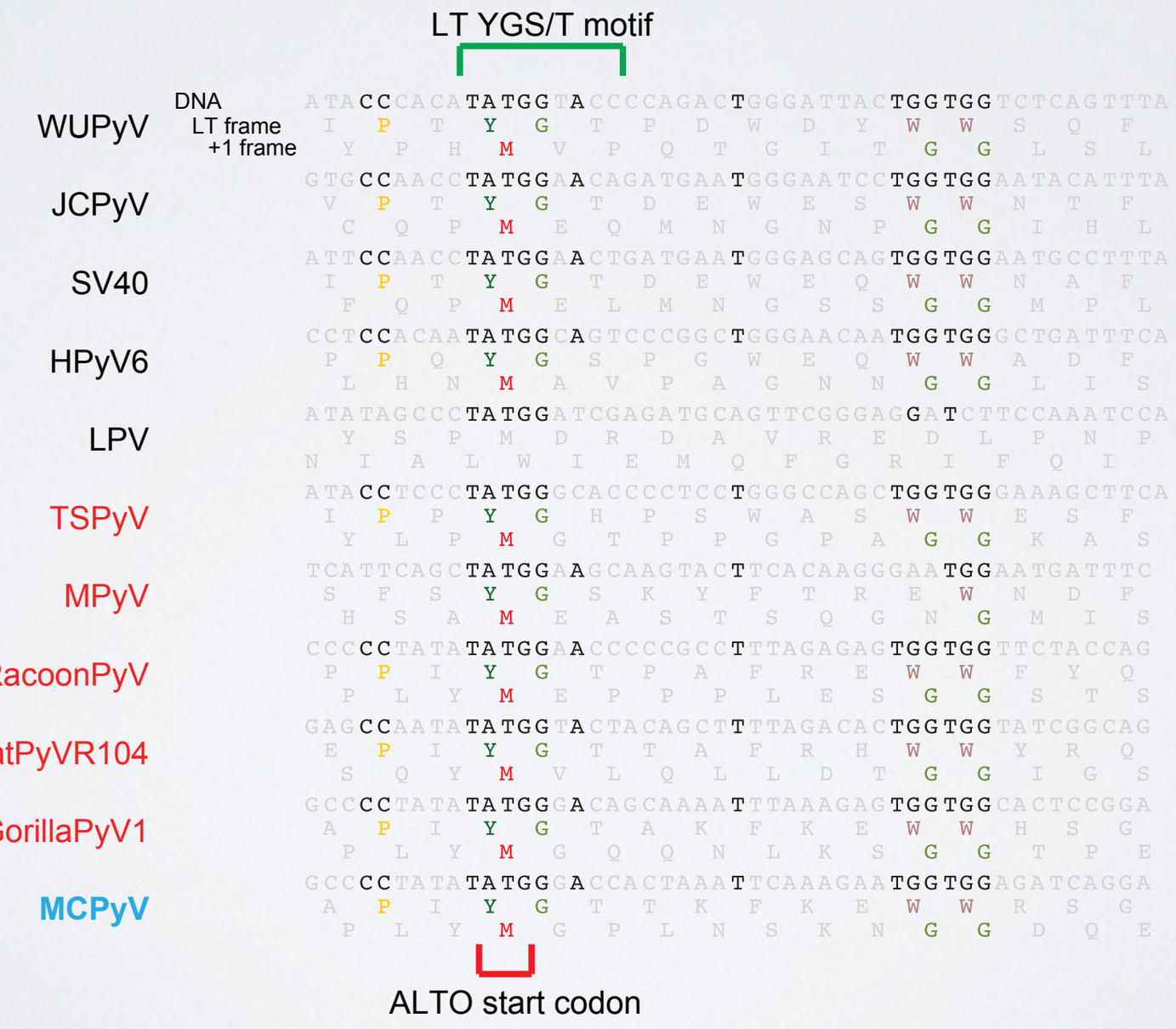
Subfunctionalization of ALTO/MT



How was this new gene born?

What was in place before ALTO was born?





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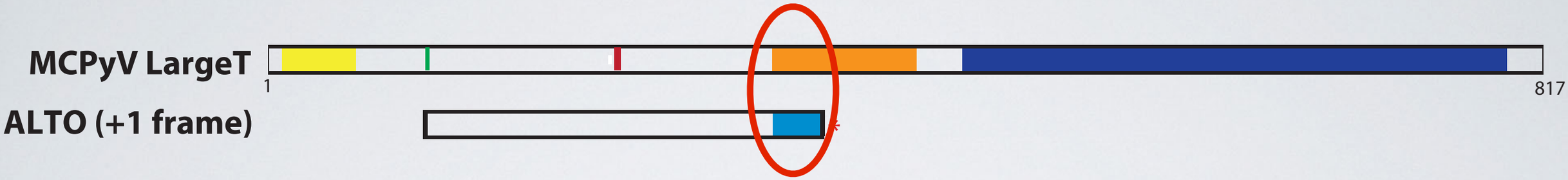
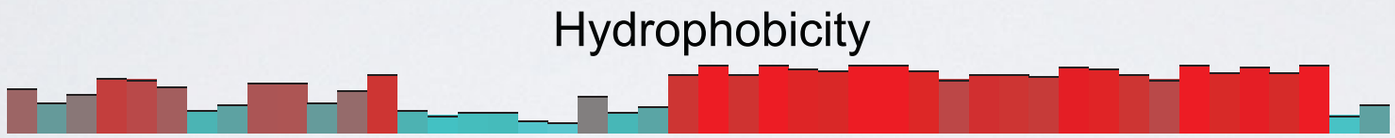
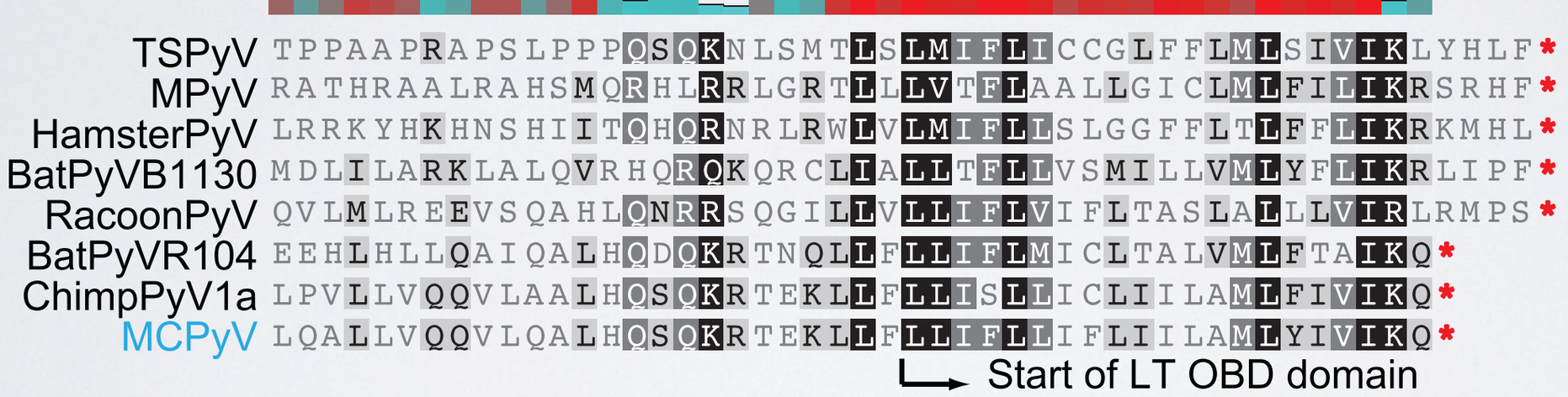
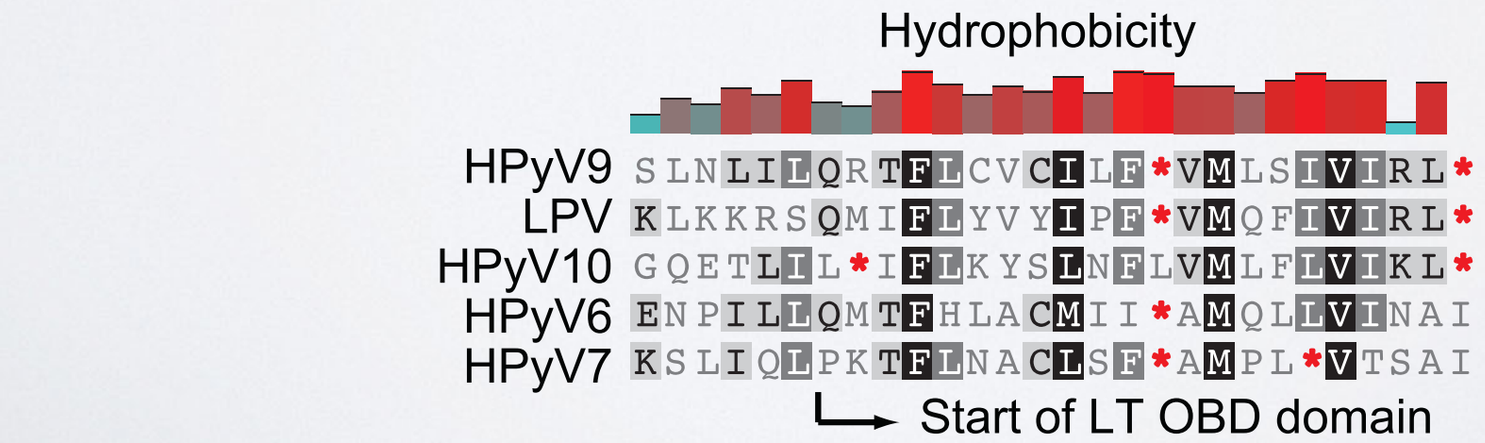




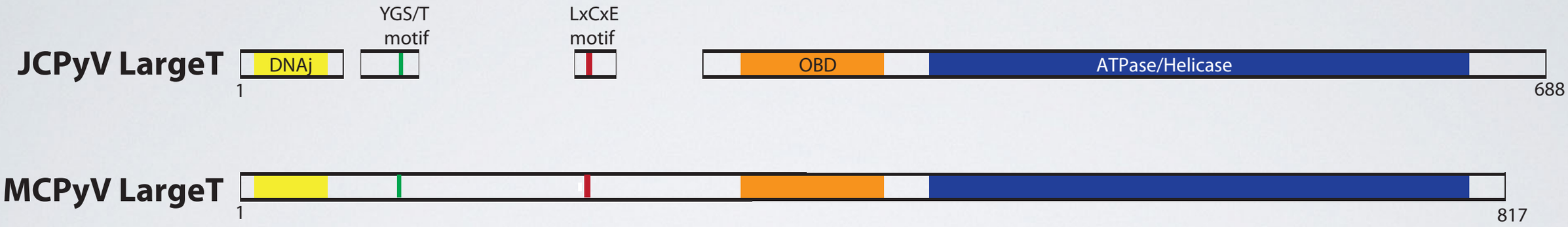


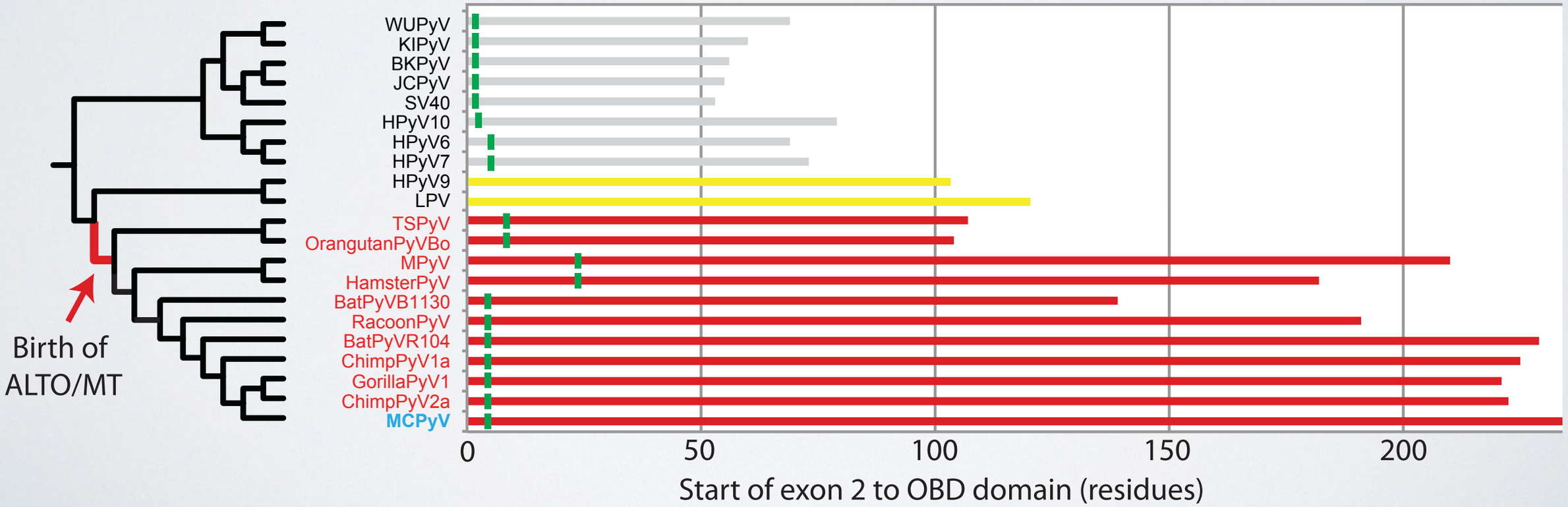


How was this new gene born?

The start codon and hydrophobic motif were in place before ALTO was born

Where did all of the extra sequence come from?





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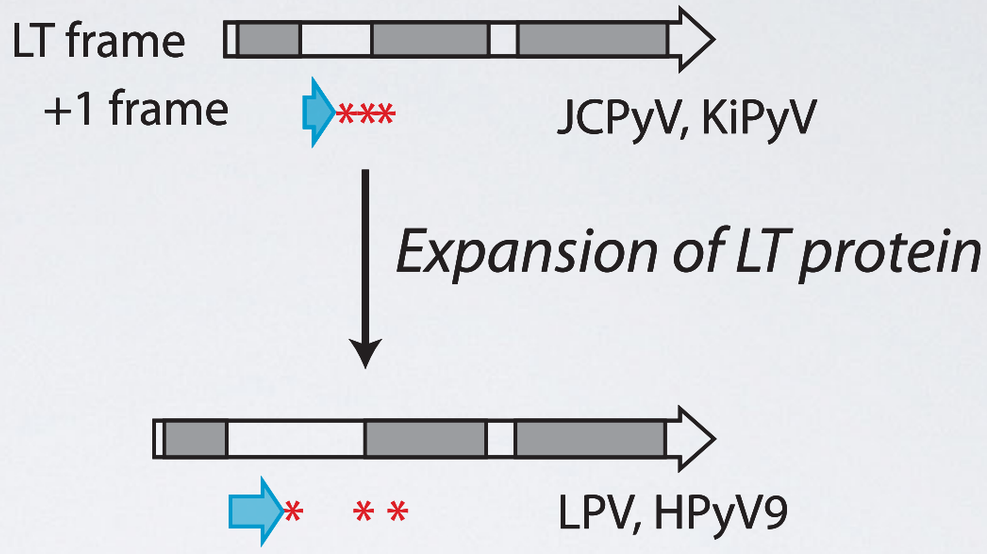

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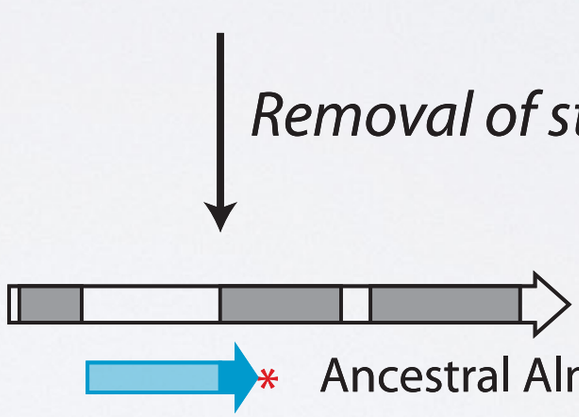
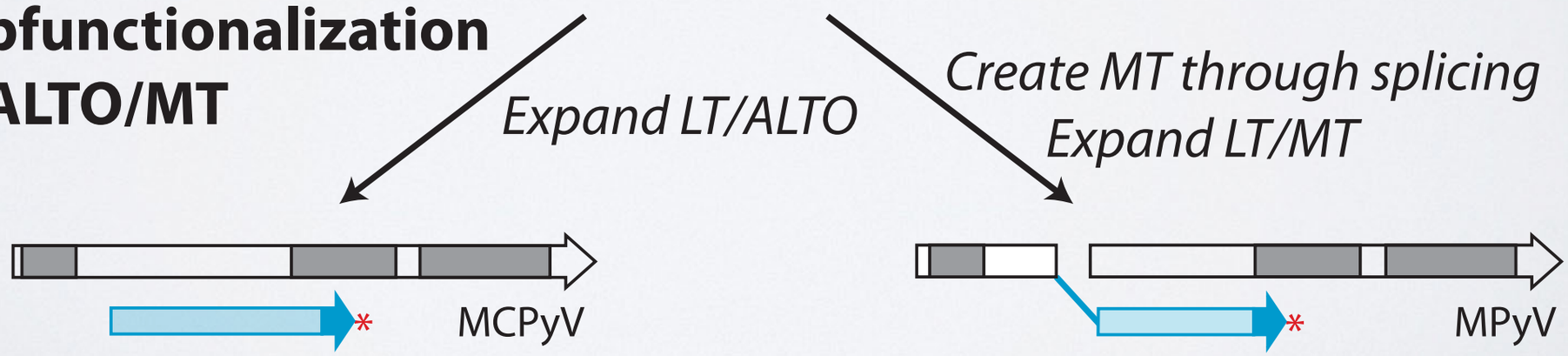
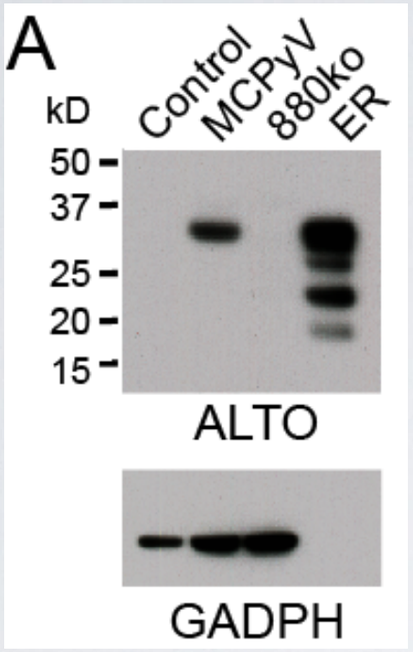
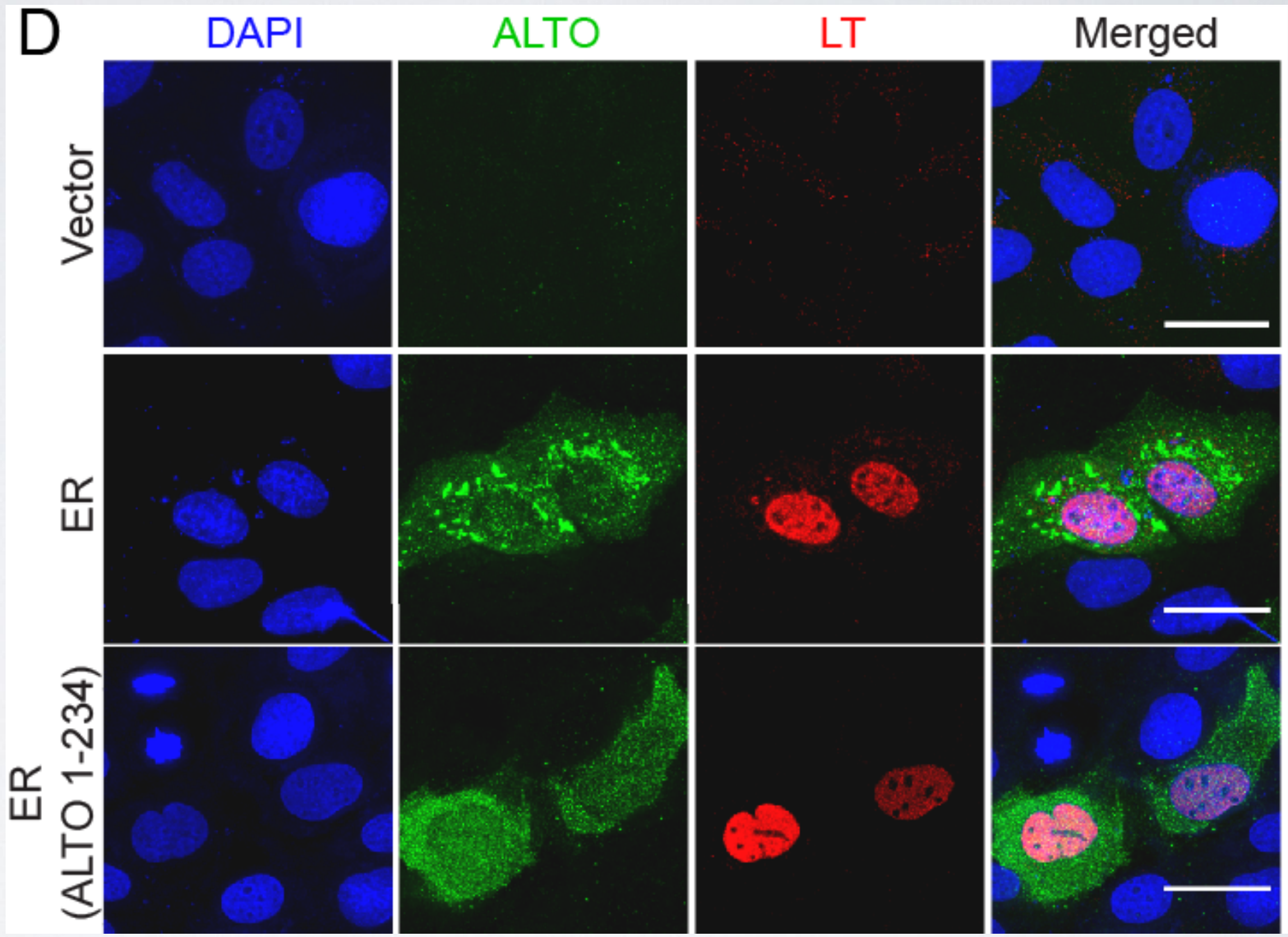


Subfunctionalization of ALTO/MT



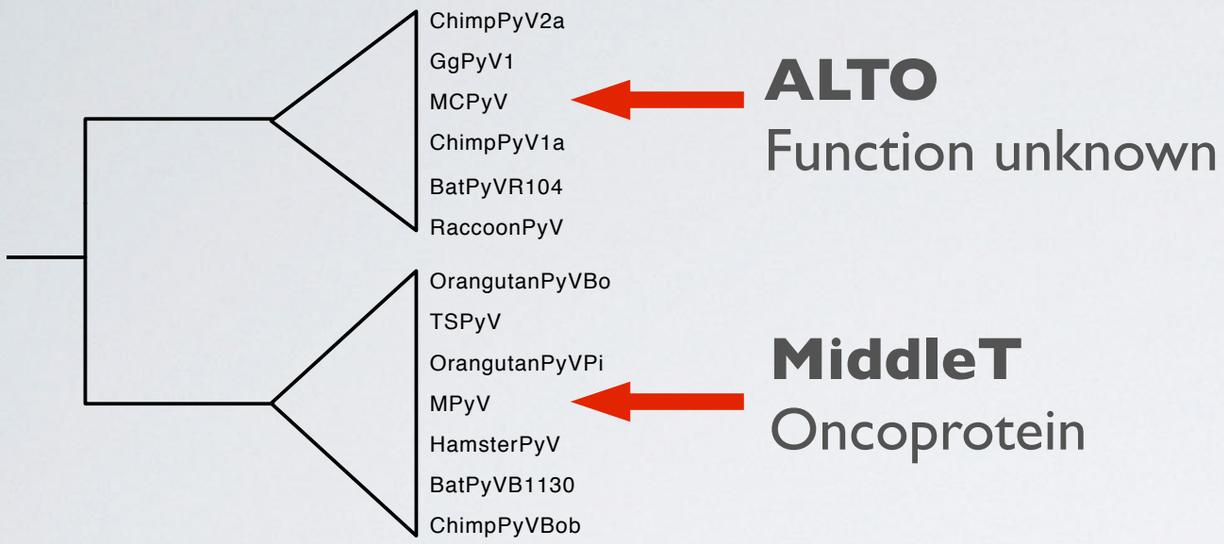
What does ALTO do?





The short answer: we don't know!

What does ALTO do?

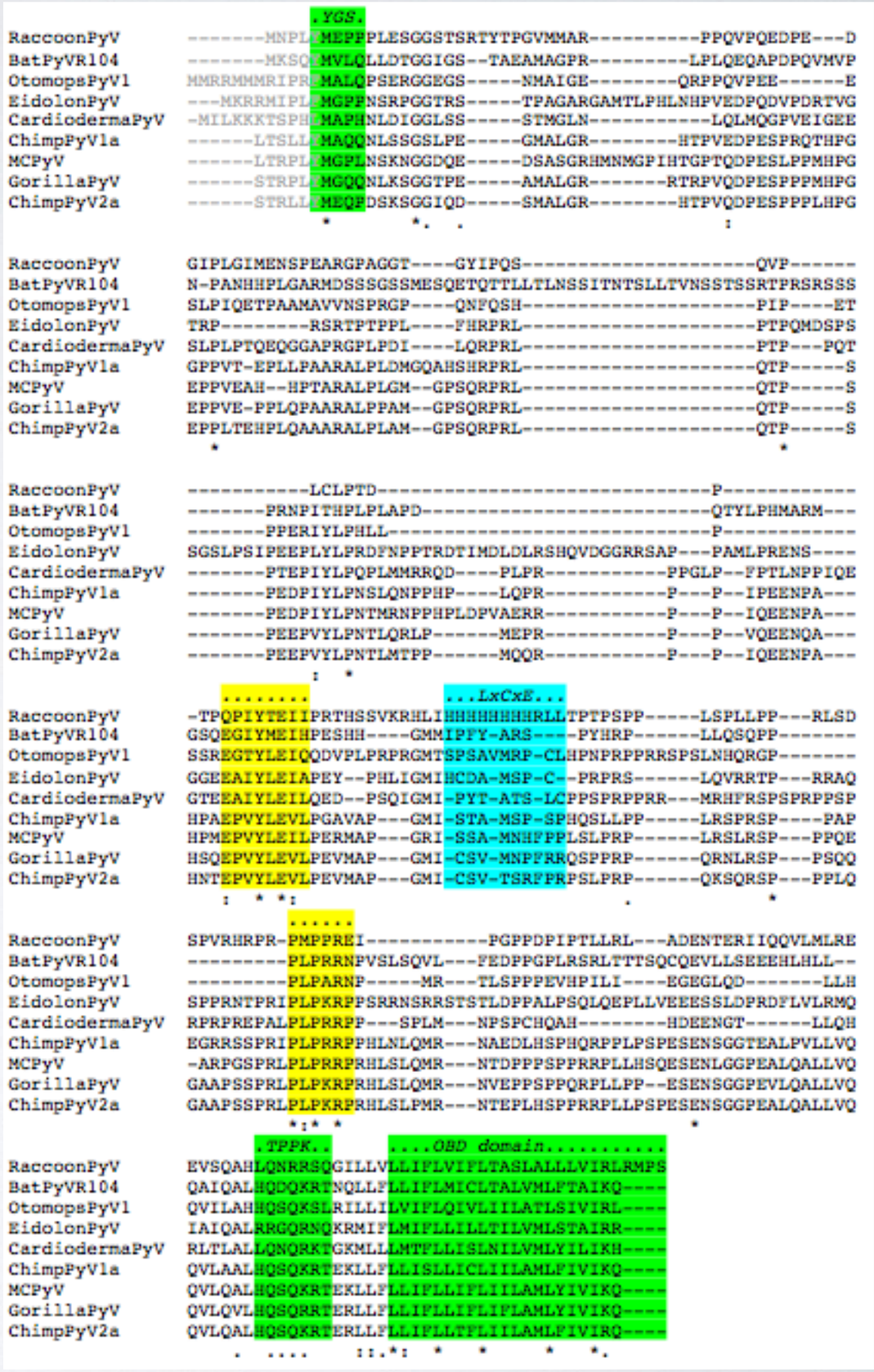


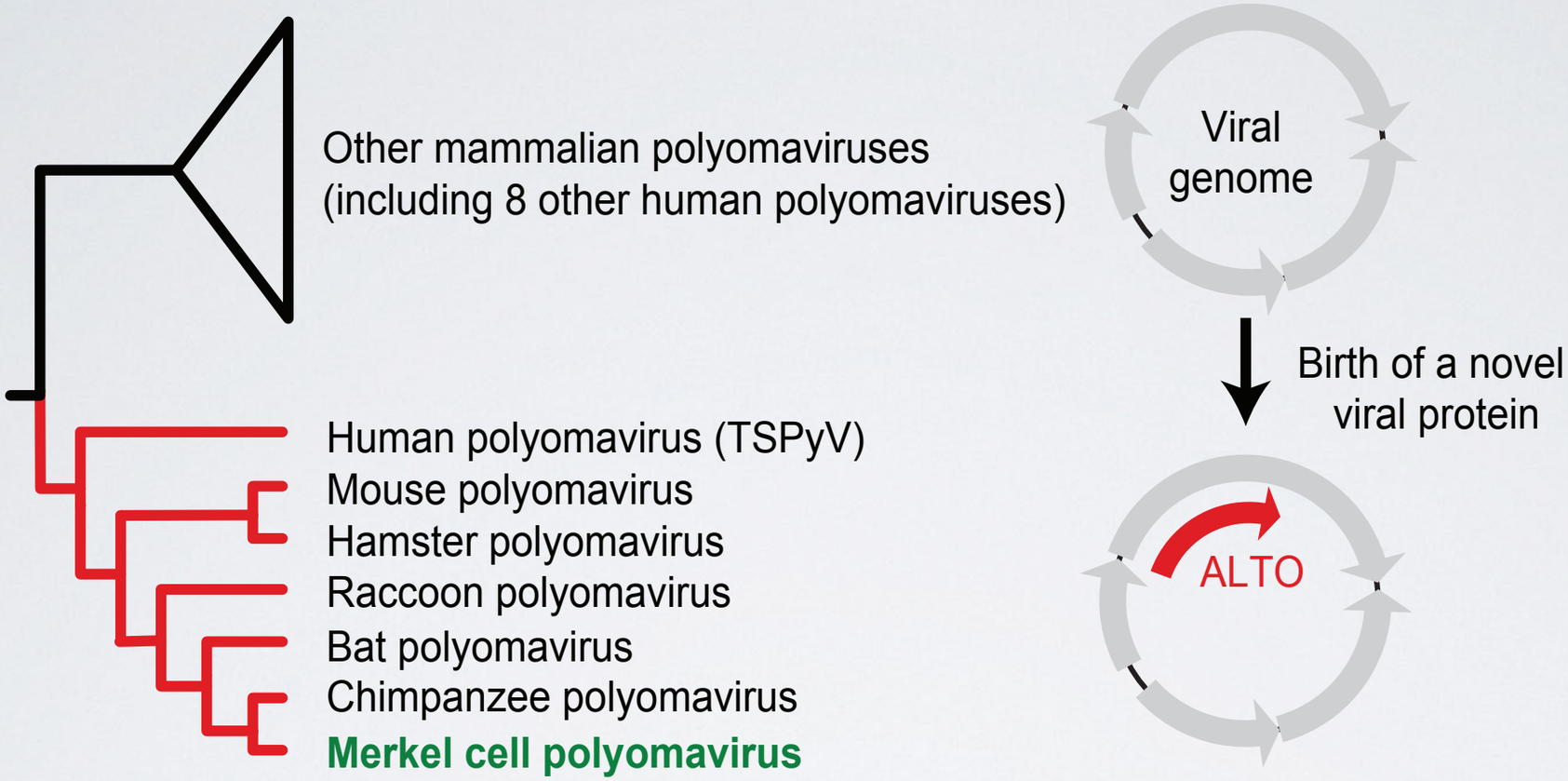
Evolutionary connection to MousePyV allows us to use MiddleT to understand ALTO

MiddleT transforms cells using short linear motifs to disrupt cell cycle control

Several of these motifs have phosphorylated Y's

Does ALTO have conserved Y motifs?
Do these motifs activate signaling cascades?

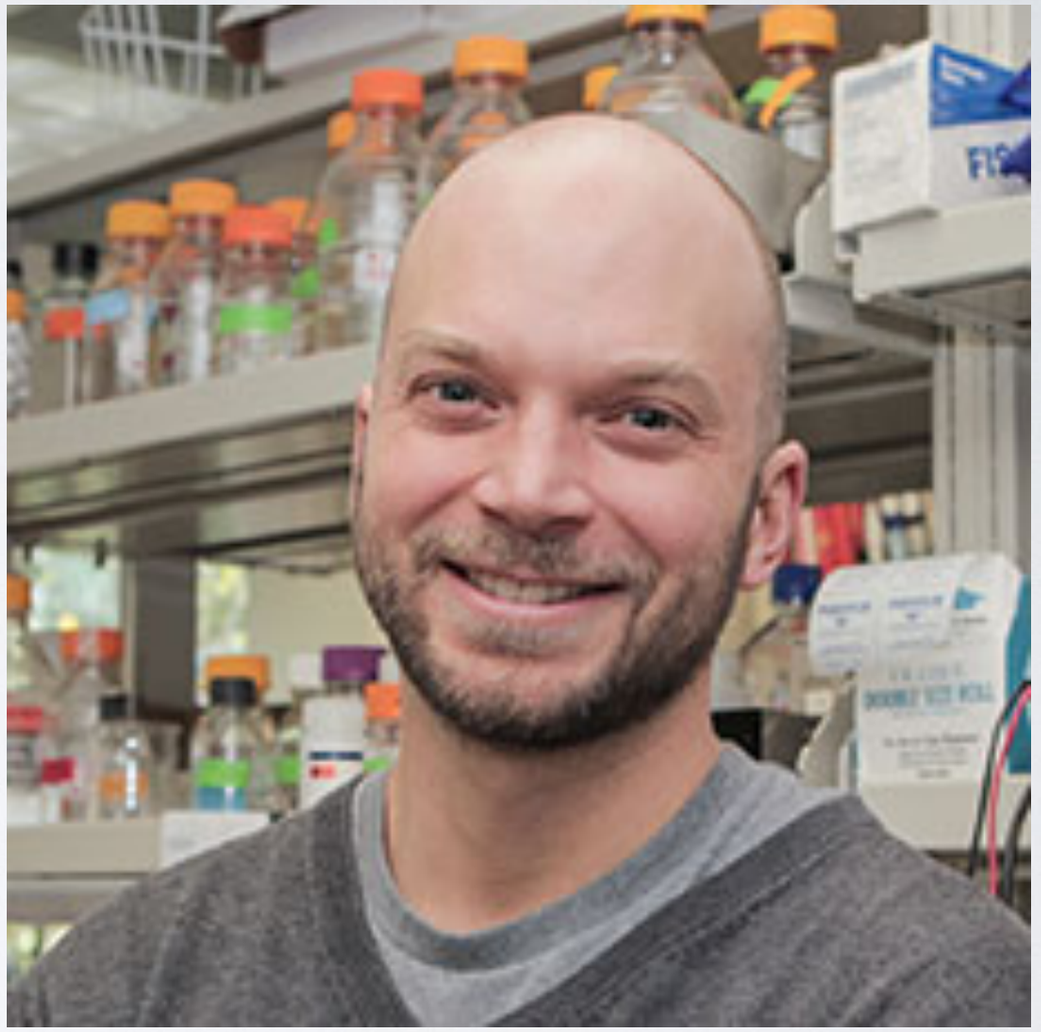



Other mammalian polyomaviruses
(including 8 other human polyomaviruses)
Human polyomavirus (TSPyV)
Mouse polyomavirus
Hamster polyomavirus
Raccoon polyomavirus
Bat polyomavirus
Chimpanzee polyomavirus
Merkel cell polyomavirus

Viral genome
Birth of a novel viral protein
ALTO

Lecture 3- Vignette 2

Deconvolution of two ancient mammalian antiviral families



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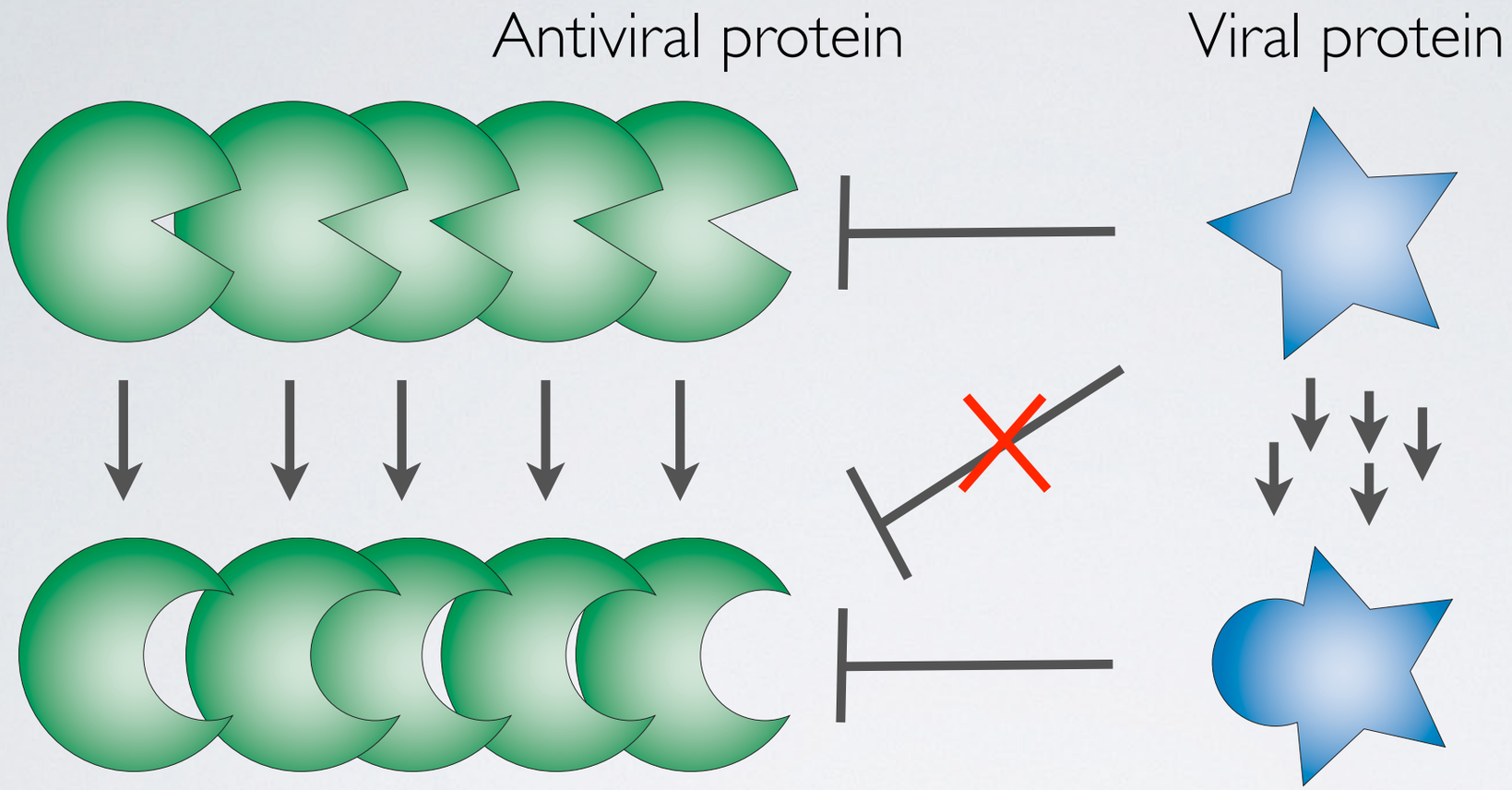
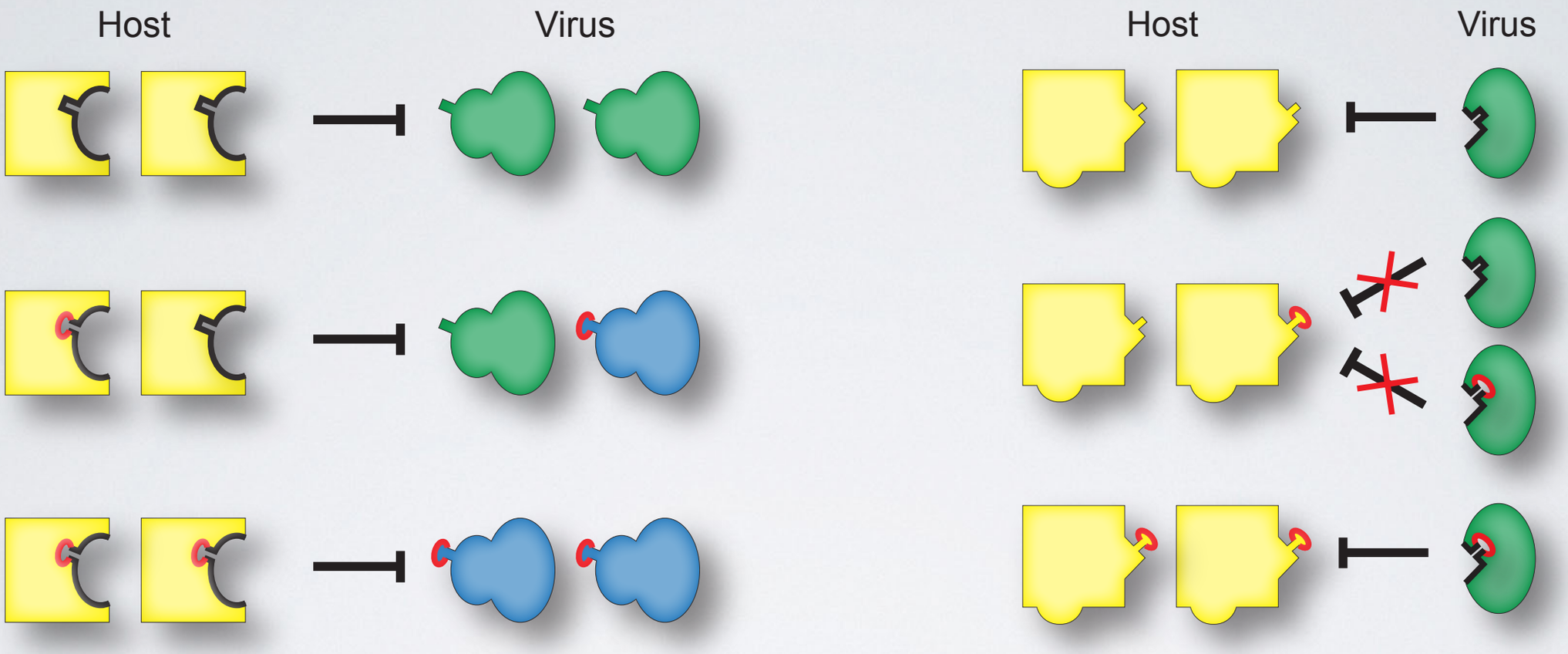
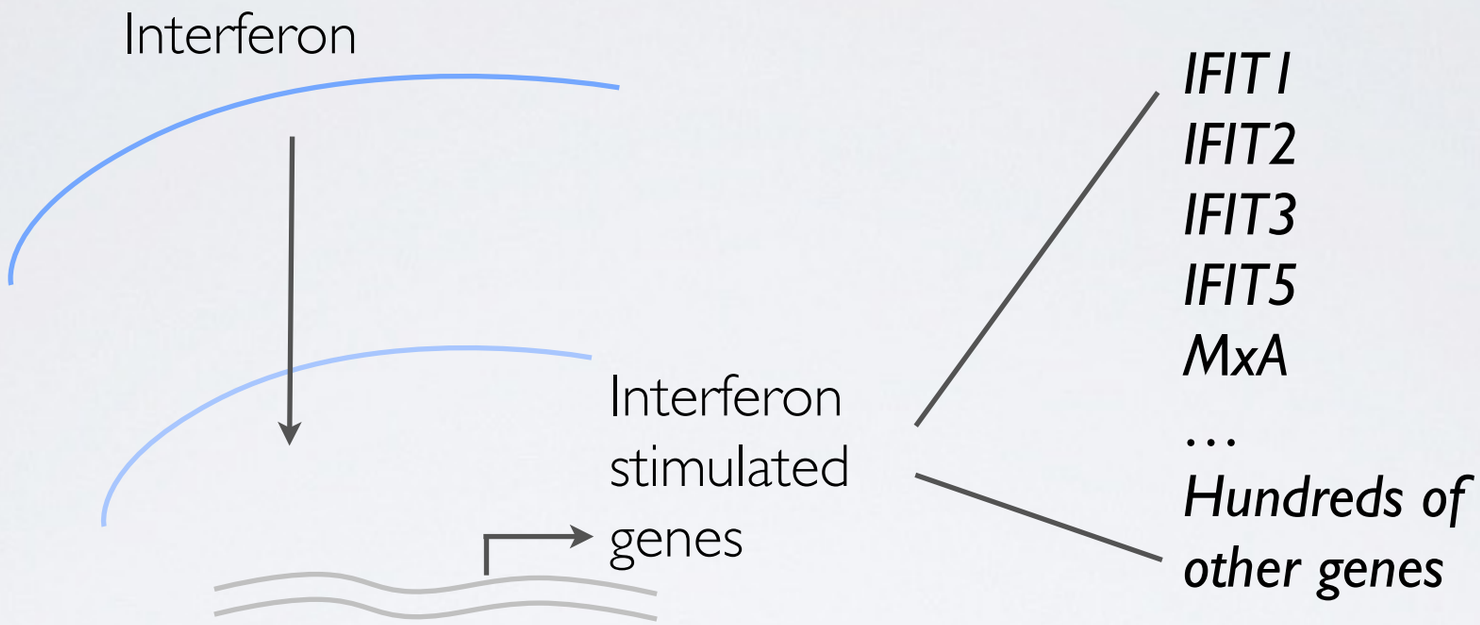

Antiviral protein
Viral protein

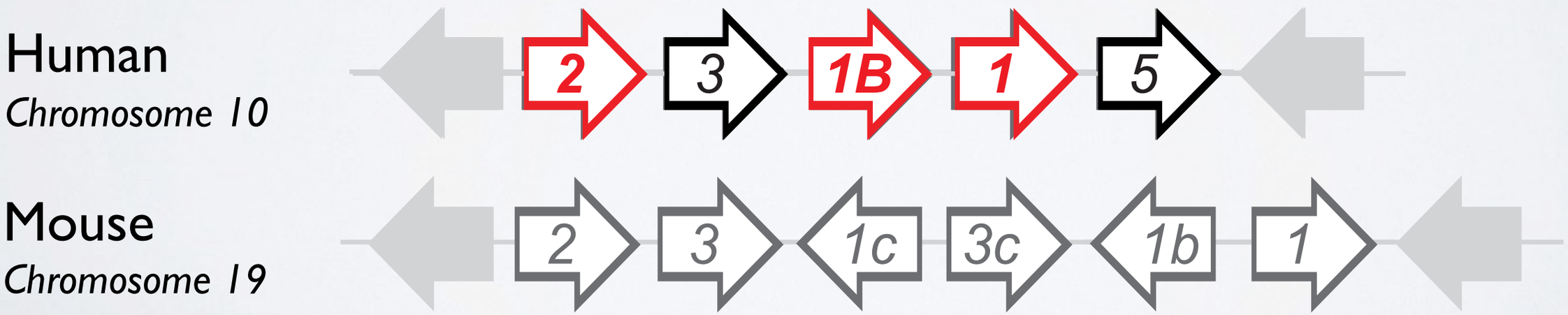
Heterozygosity or gene duplications provides host genomes with an advantage against viruses



IFIT genes: a case-study in diversity

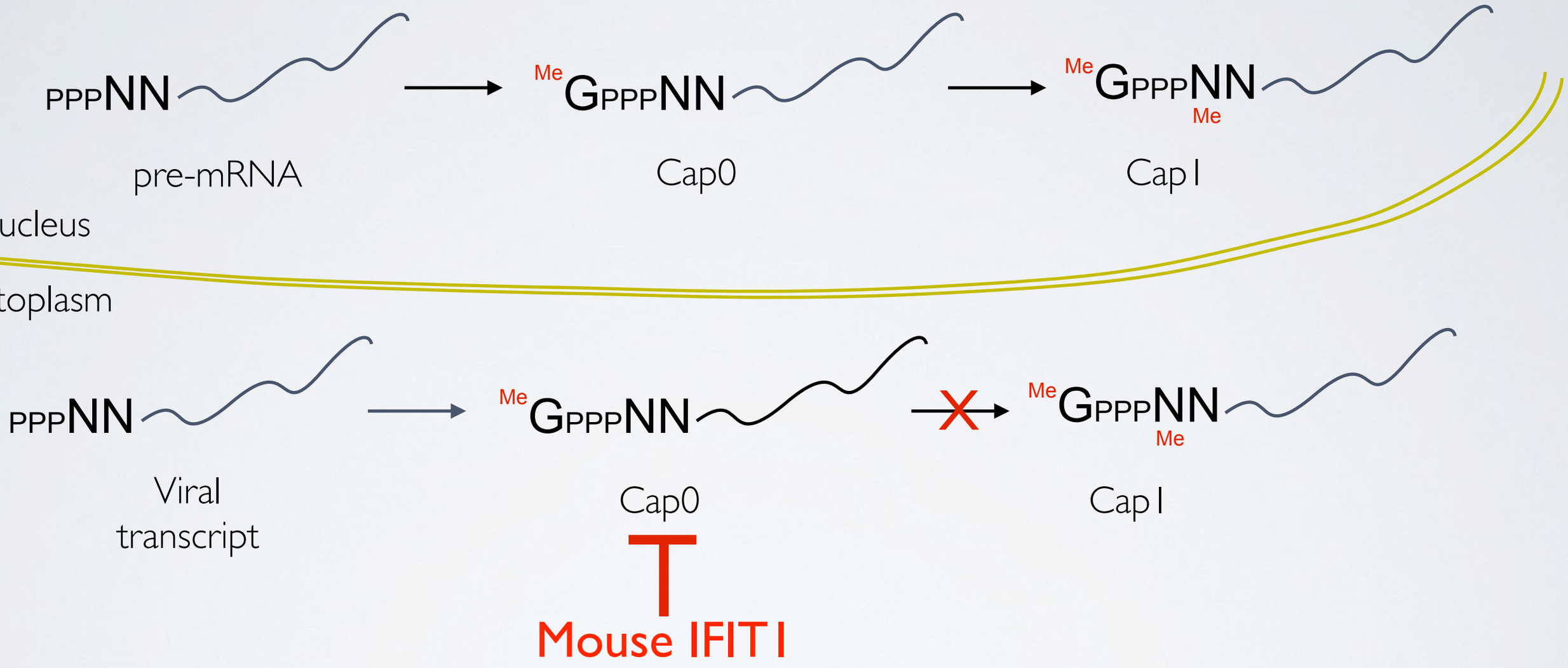


IFIT: Interferon-induced with tetratricopeptide (TPR) repeats



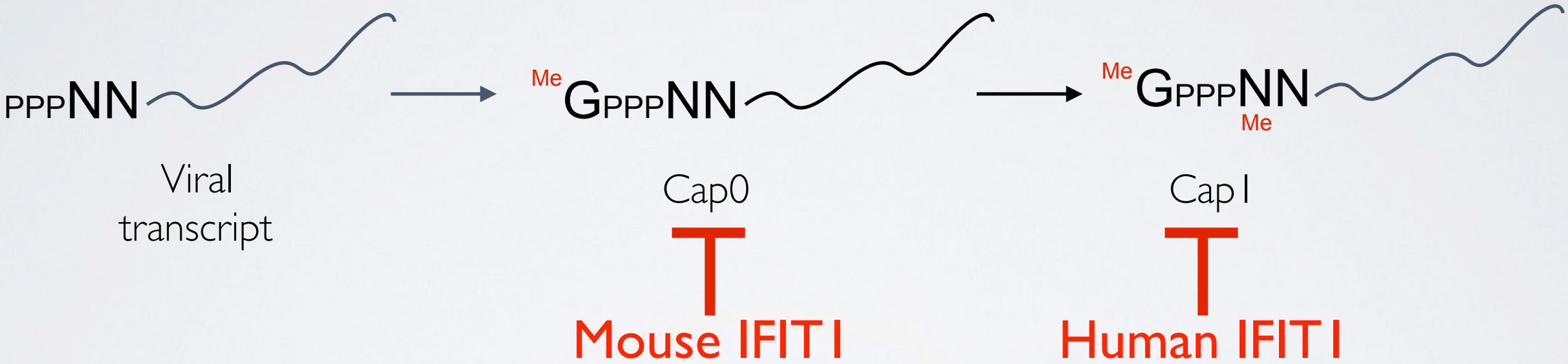
What are the functional consequences of IFIT evolution?

IFIT1 distinguishes 'self' from 'non-self'



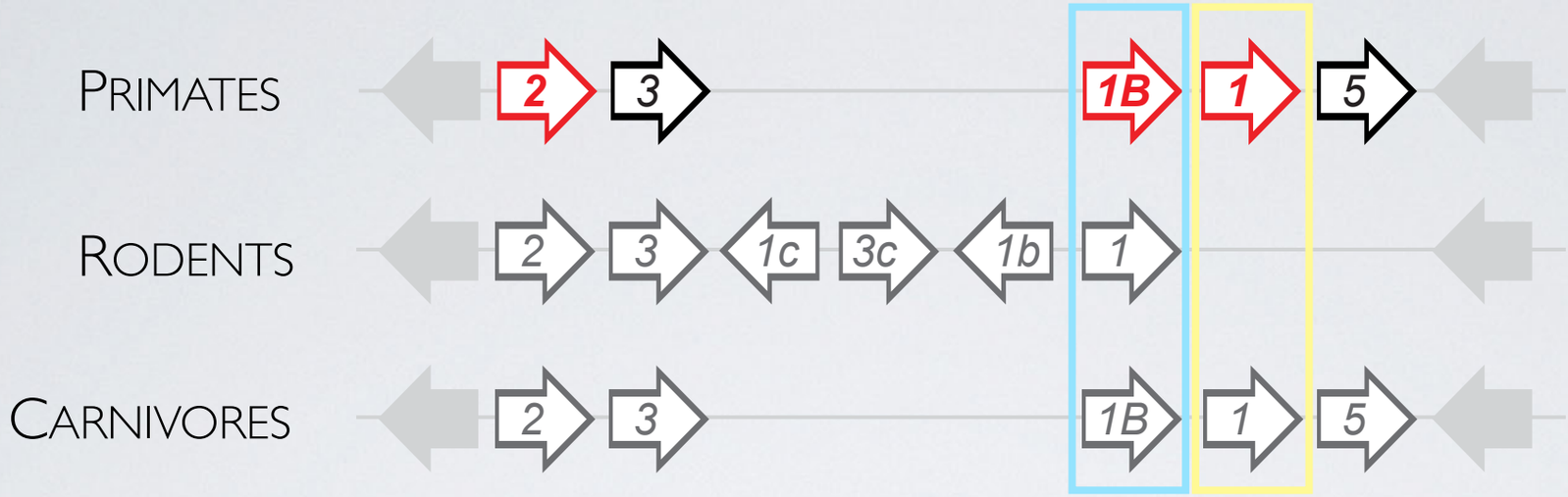
Daffis et al, Nature, 2010

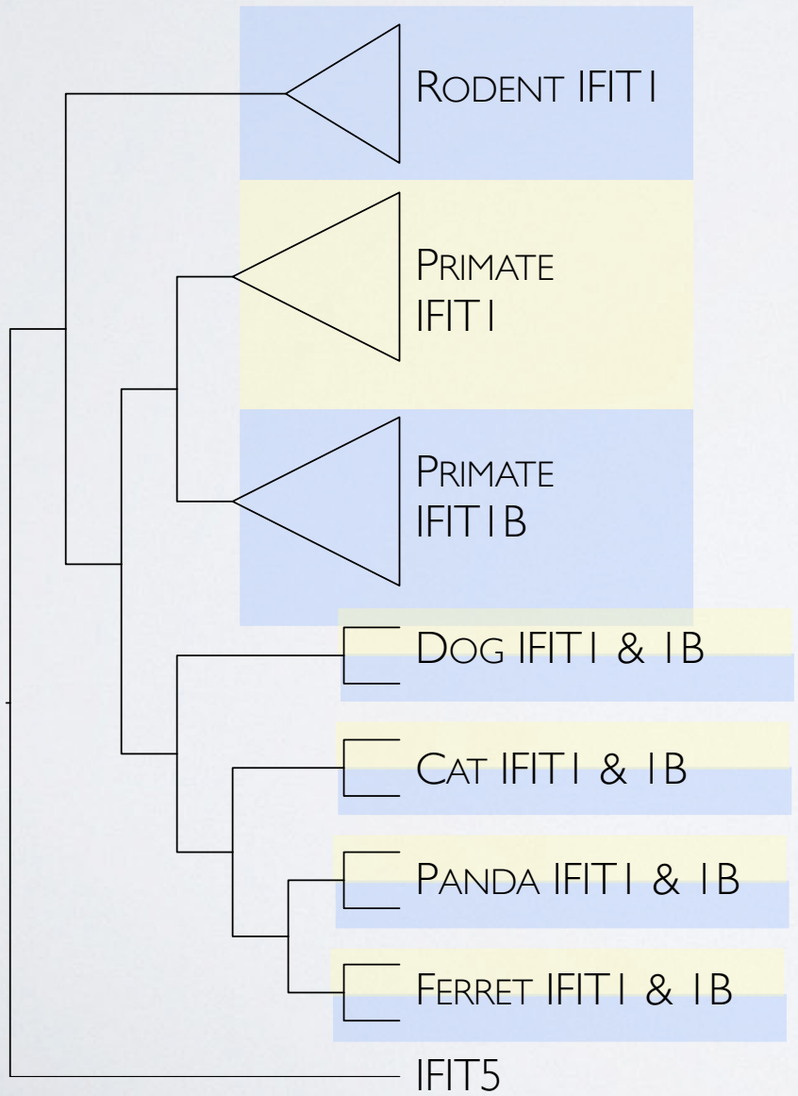
A discrepancy in IFIT1 function



Pichlmair, 2011
Andrejeva, 2013

IFIT phylogeny violates mammalian phylogeny



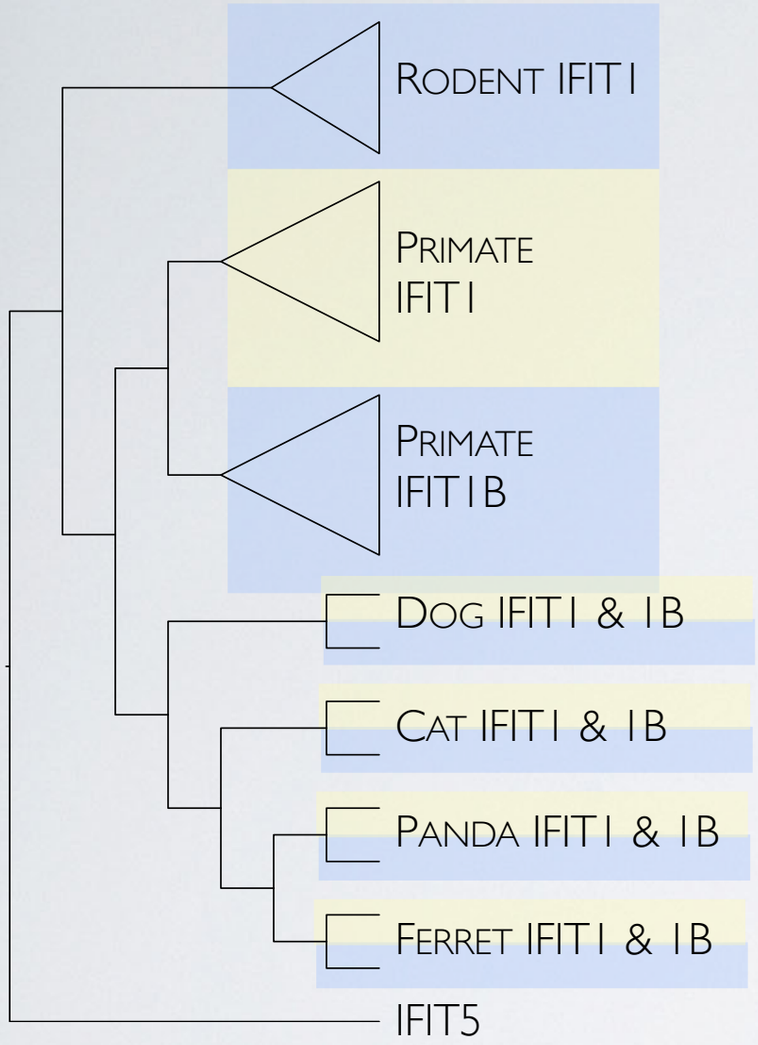


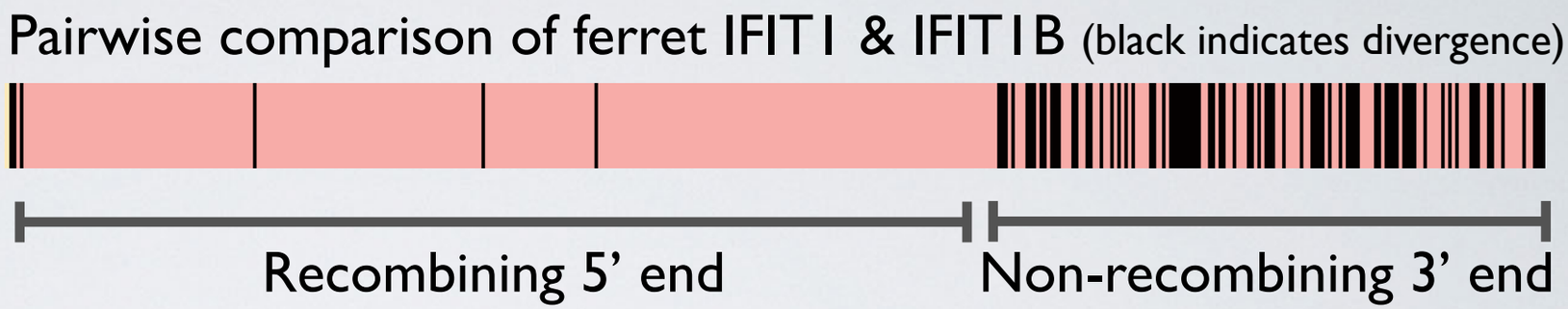
Whole gene phylogeny of IFIT1 and IFIT1B

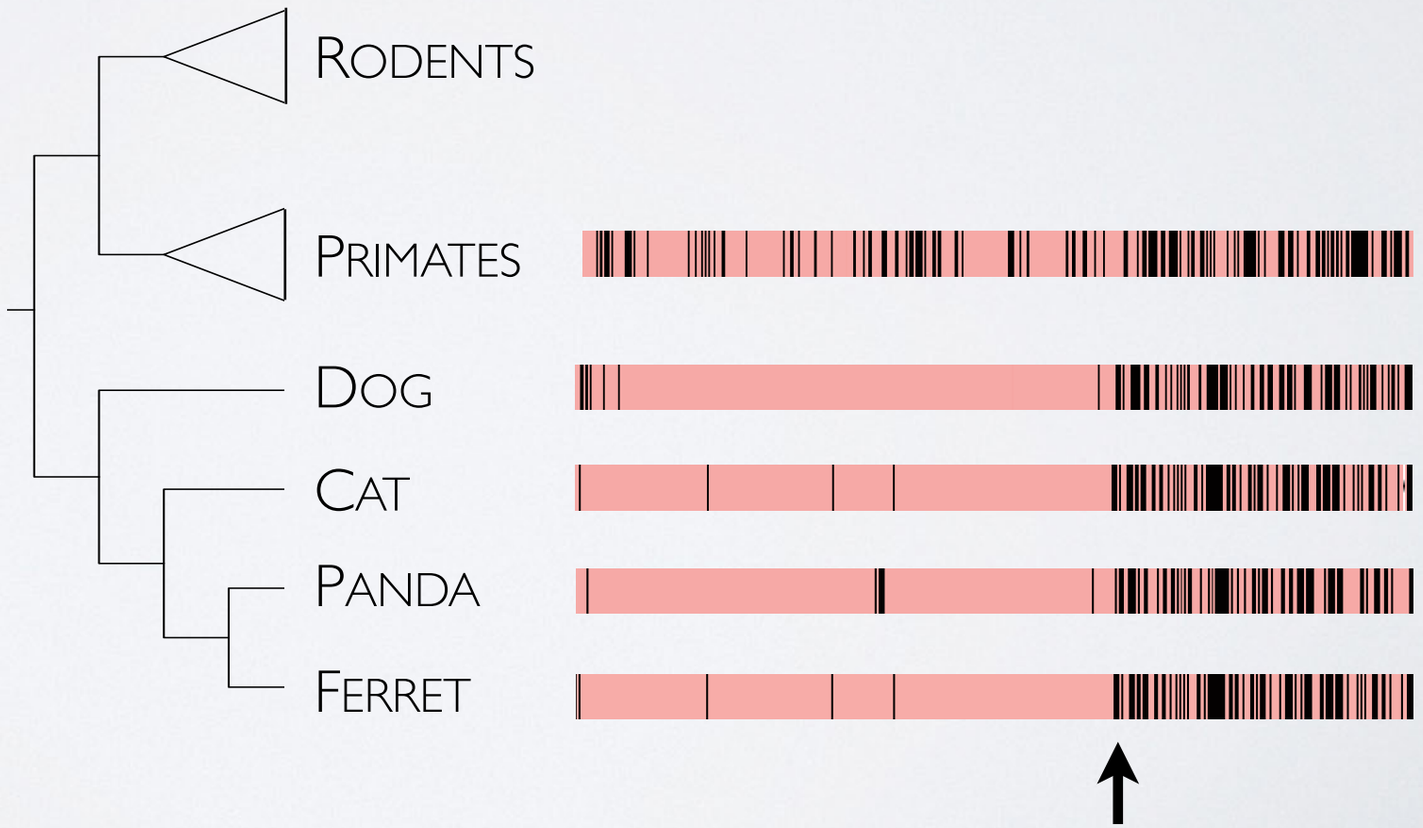
Suggests many independent duplications
(into the same chromosomal location)

Is there another possible explanation for this phylogeny?

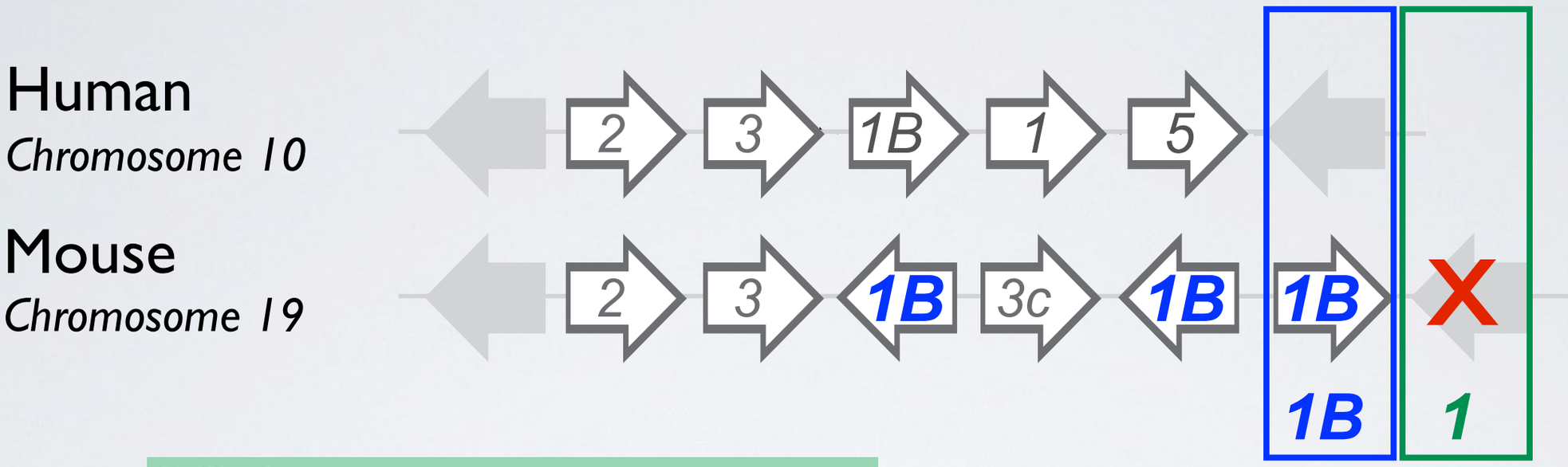
Recurrent recombination among IFIT1 parallels in mammals

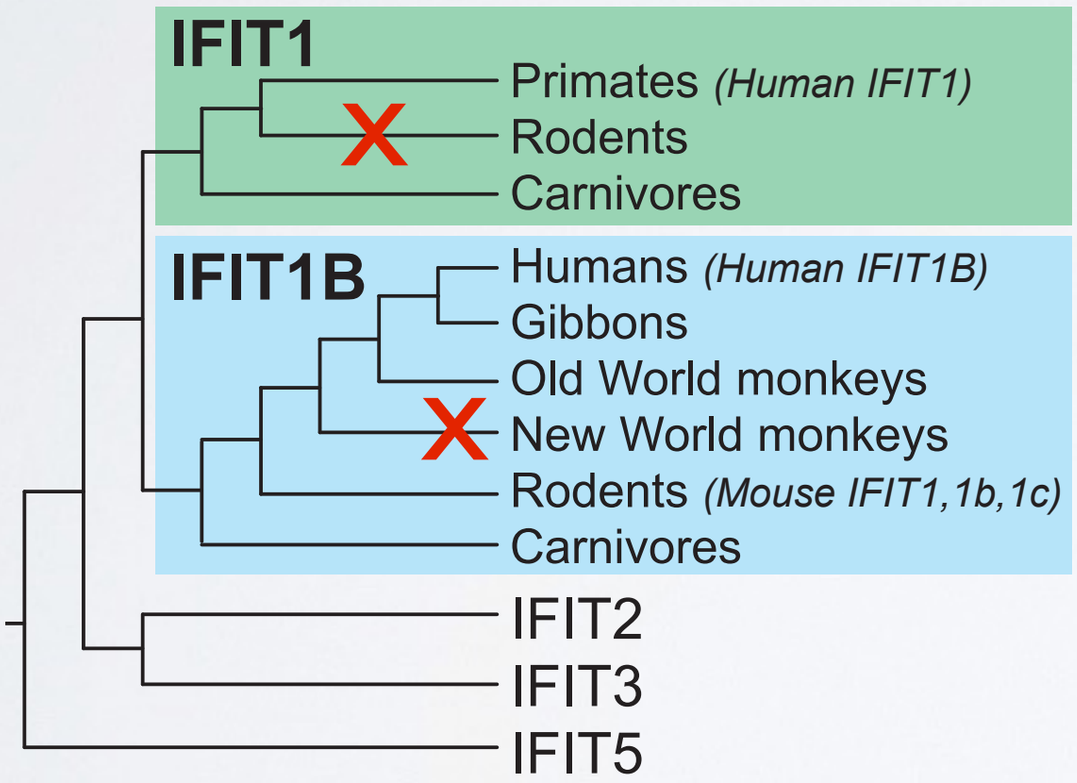






TWO ancient families of IFIT1 genes



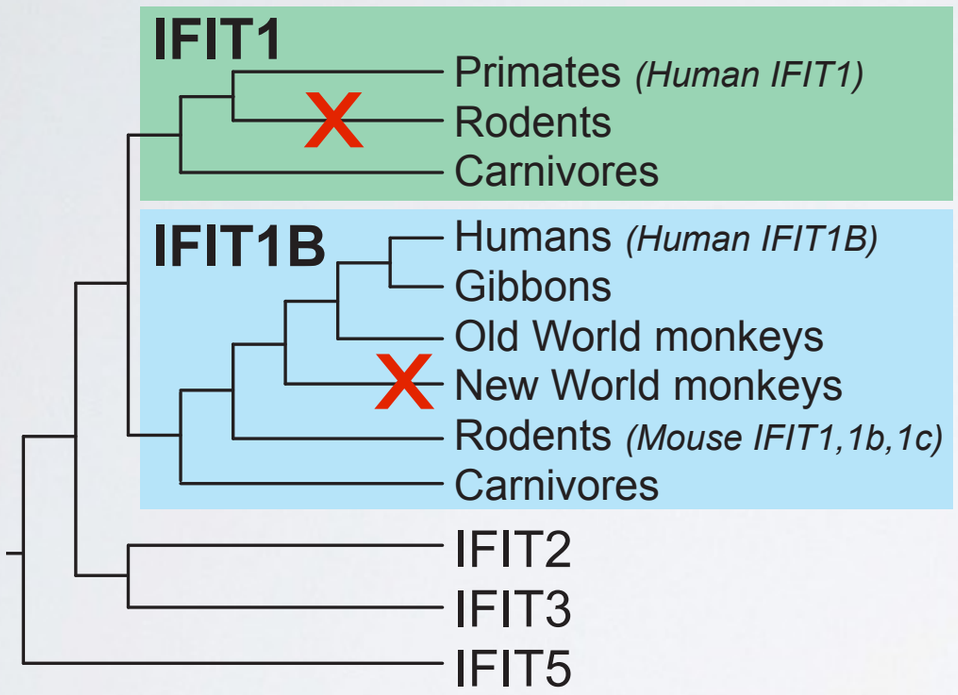


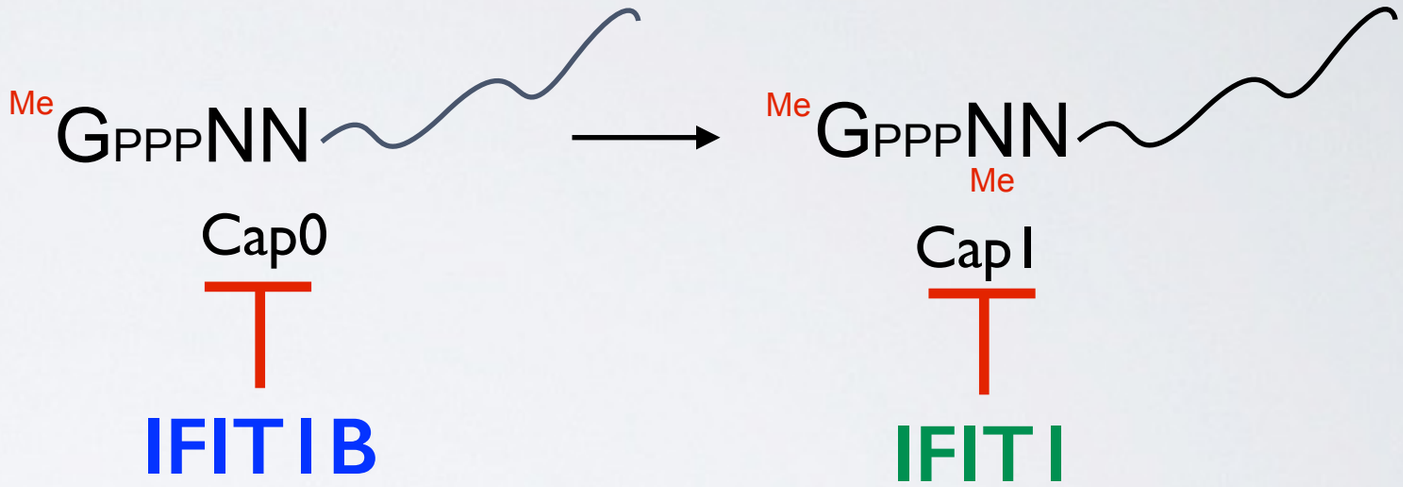
Missing IFIT1:
Mice, rats, hamsters, guinea pigs

Missing IFIT1B:
Chimpanzees, New World monkeys, pigs, cows

IFIT1 and IFIT1B have been diverging for >100 million years!
But many species only have one or the other!

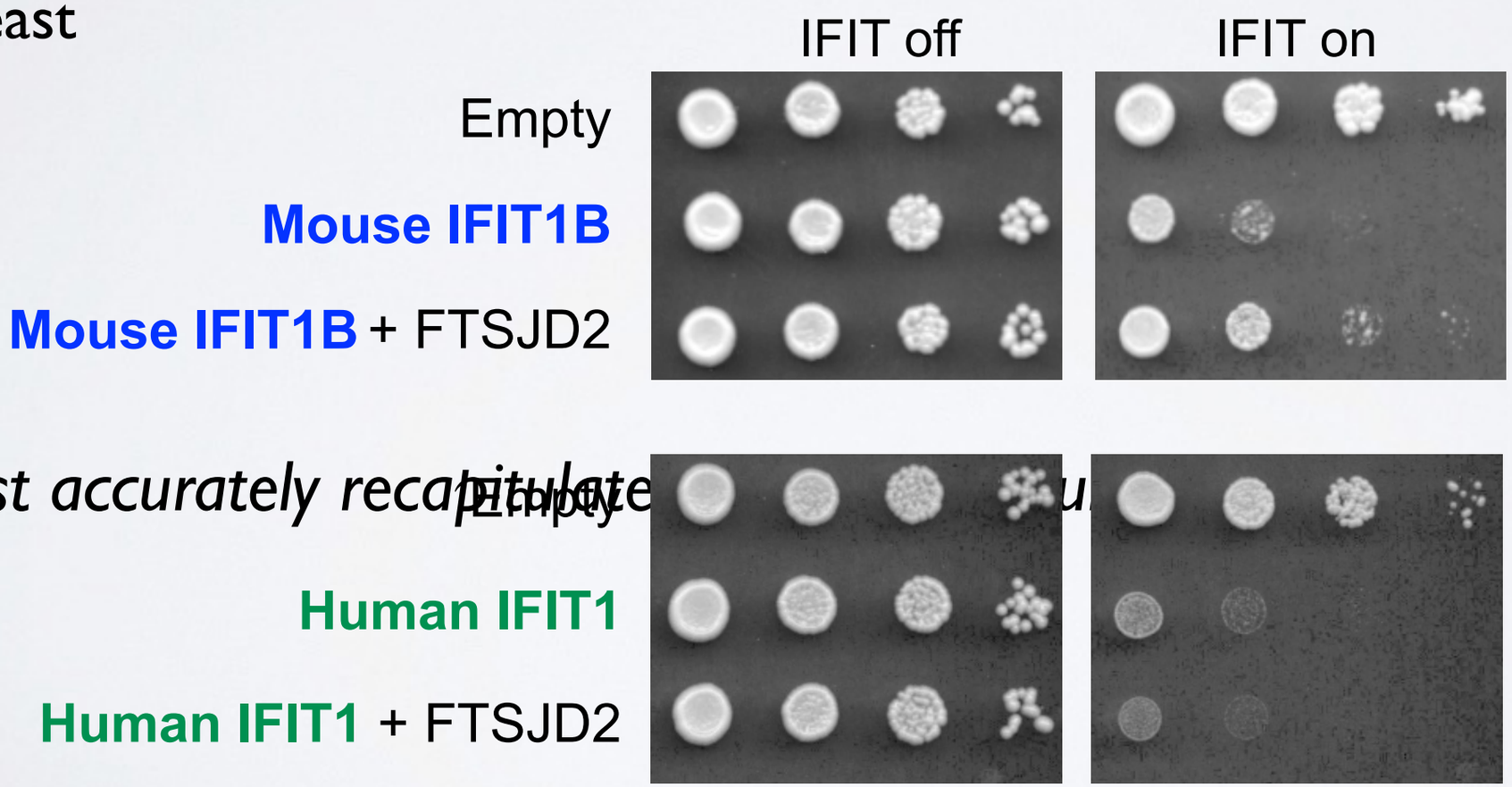
FUNCTIONAL DIVERGENCE OF IFIT1 PROTEINS?





Want to test IFIT activity independent of rest of innate immune system

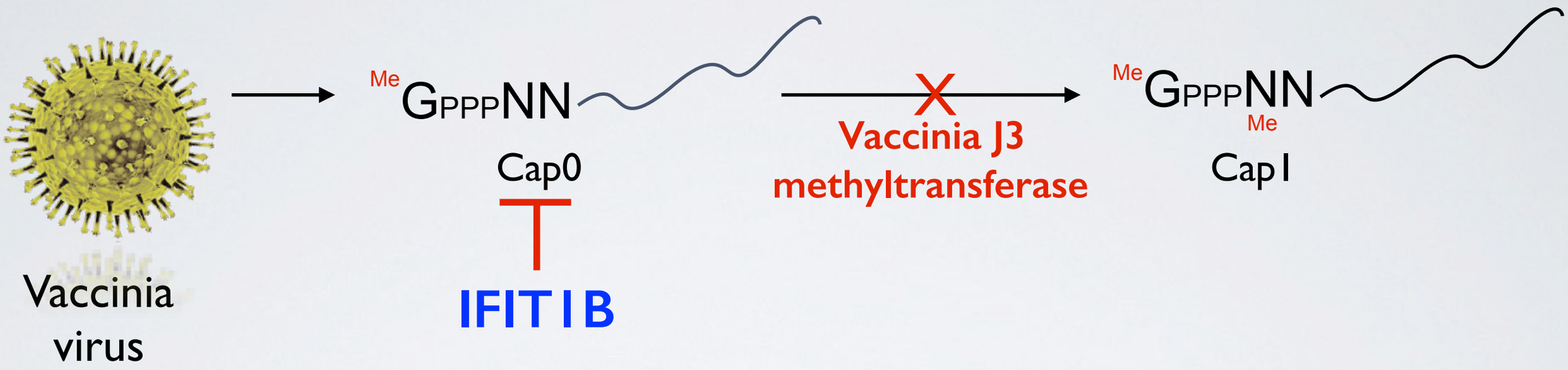
A NOVEL YEAST ASSAY FOR IFIT FUNCTION



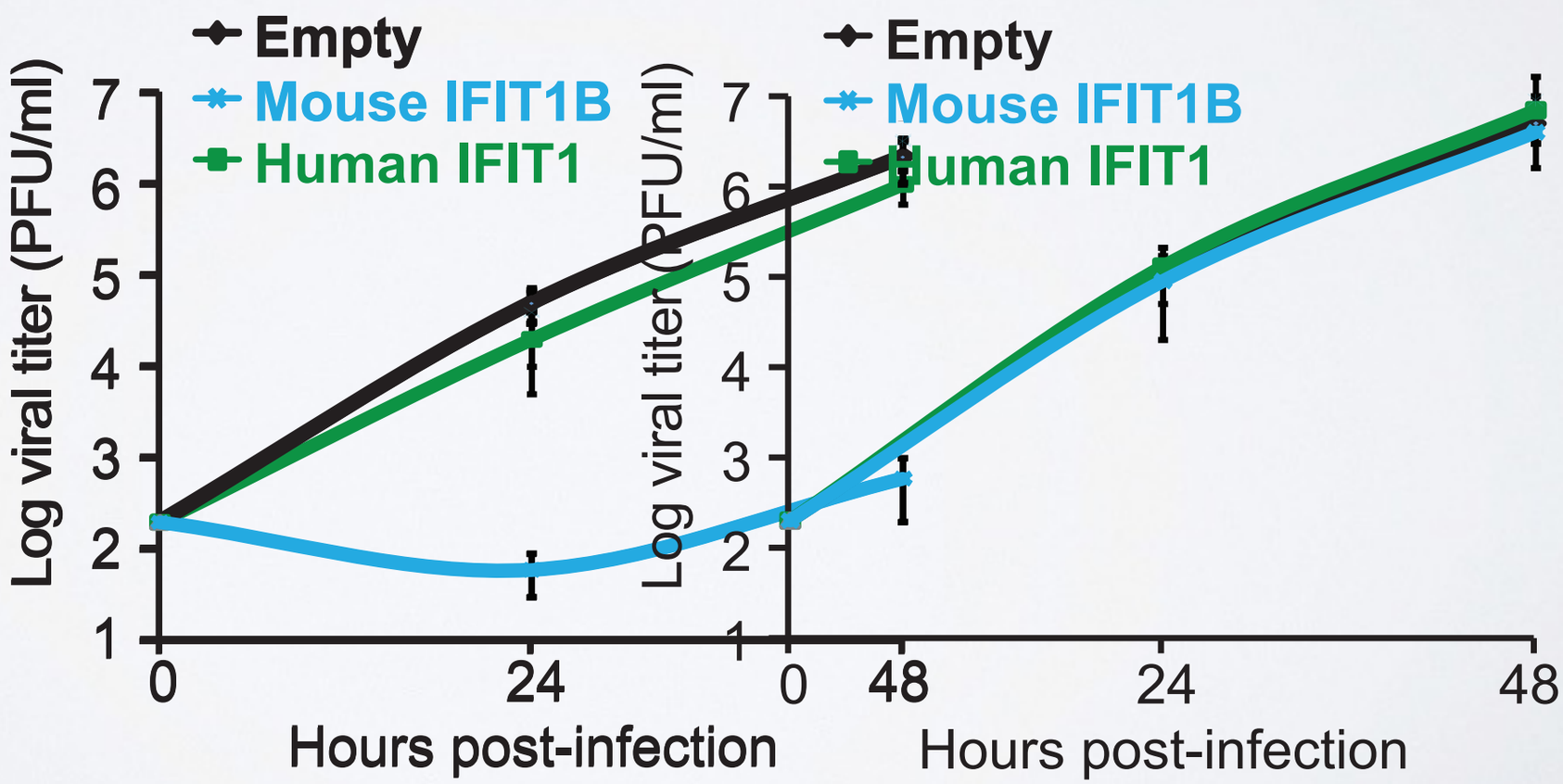
Yeast accurately recapitulate

IFIT1 recognizes a different molecular pattern to arrest yeast growth

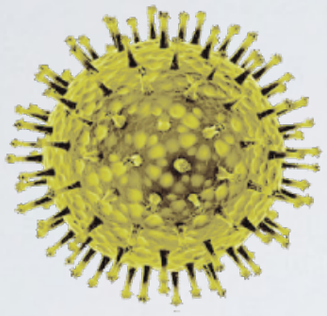
IFIT-MEDIATED ANTIVIRAL RESTRICTION



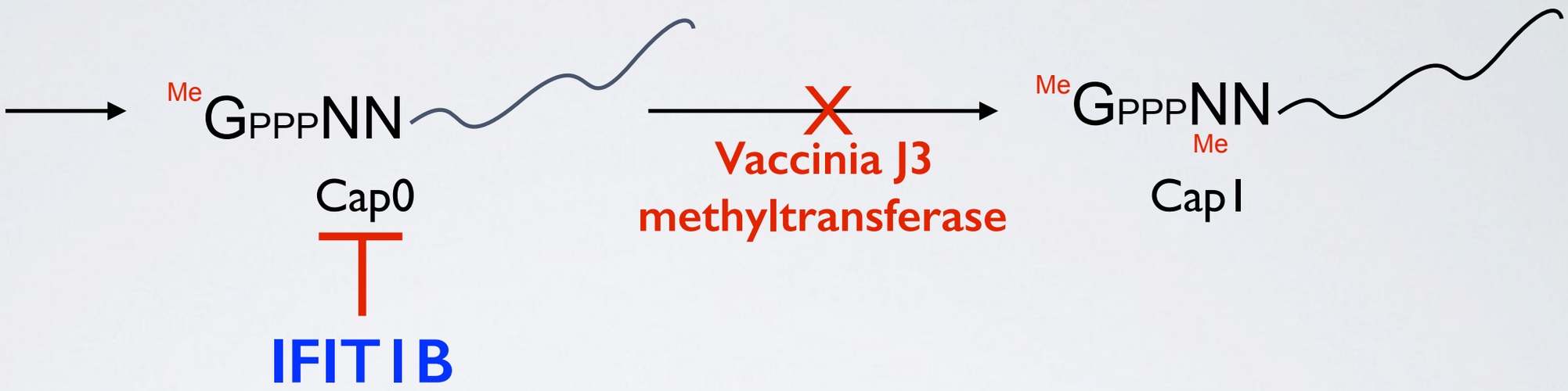
Mutant vaccinia virus (Cap0) ***WT vaccinia virus (Cap1)***

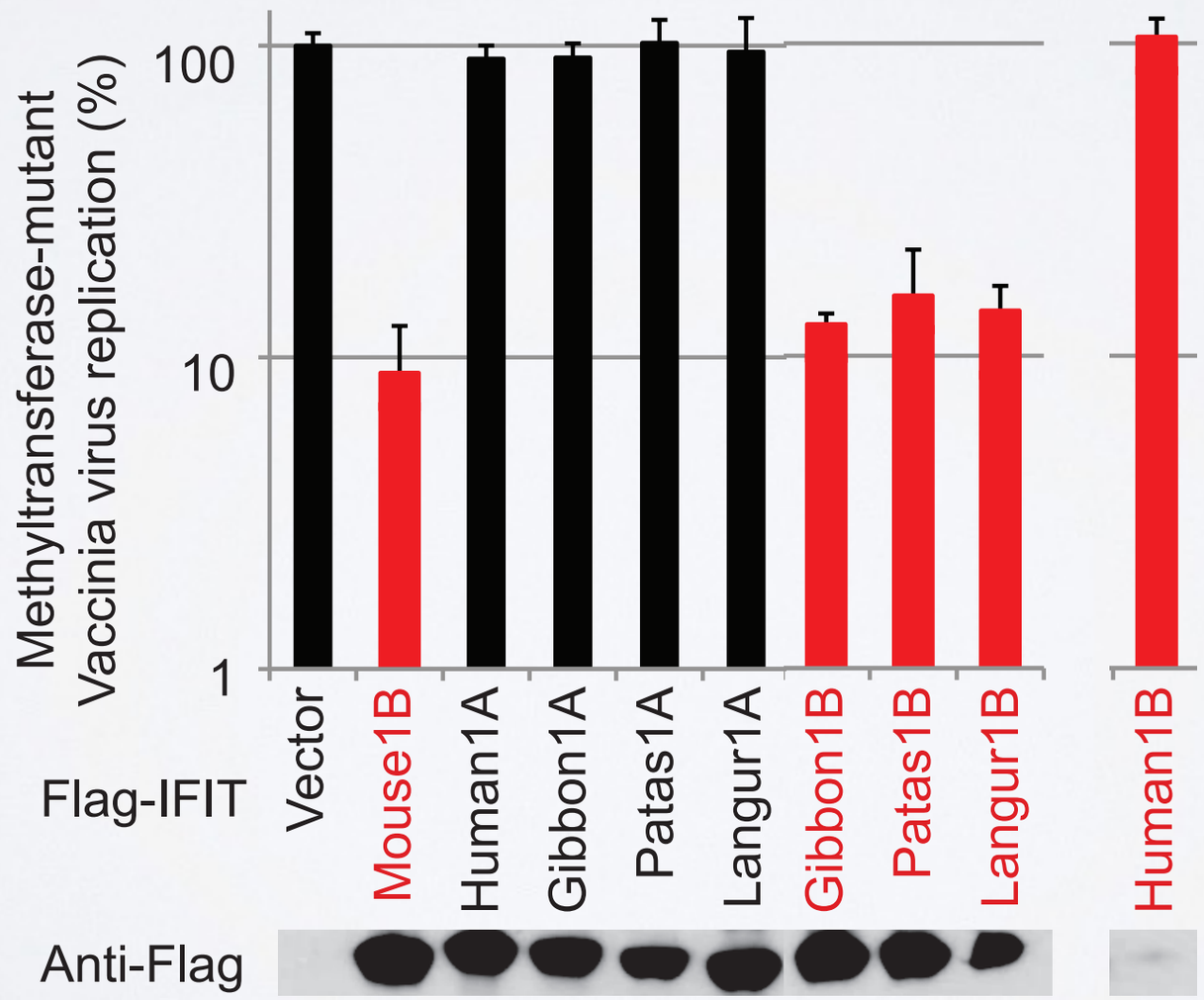


IFIT-MEDIATED ANTIVIRAL RESTRICTION

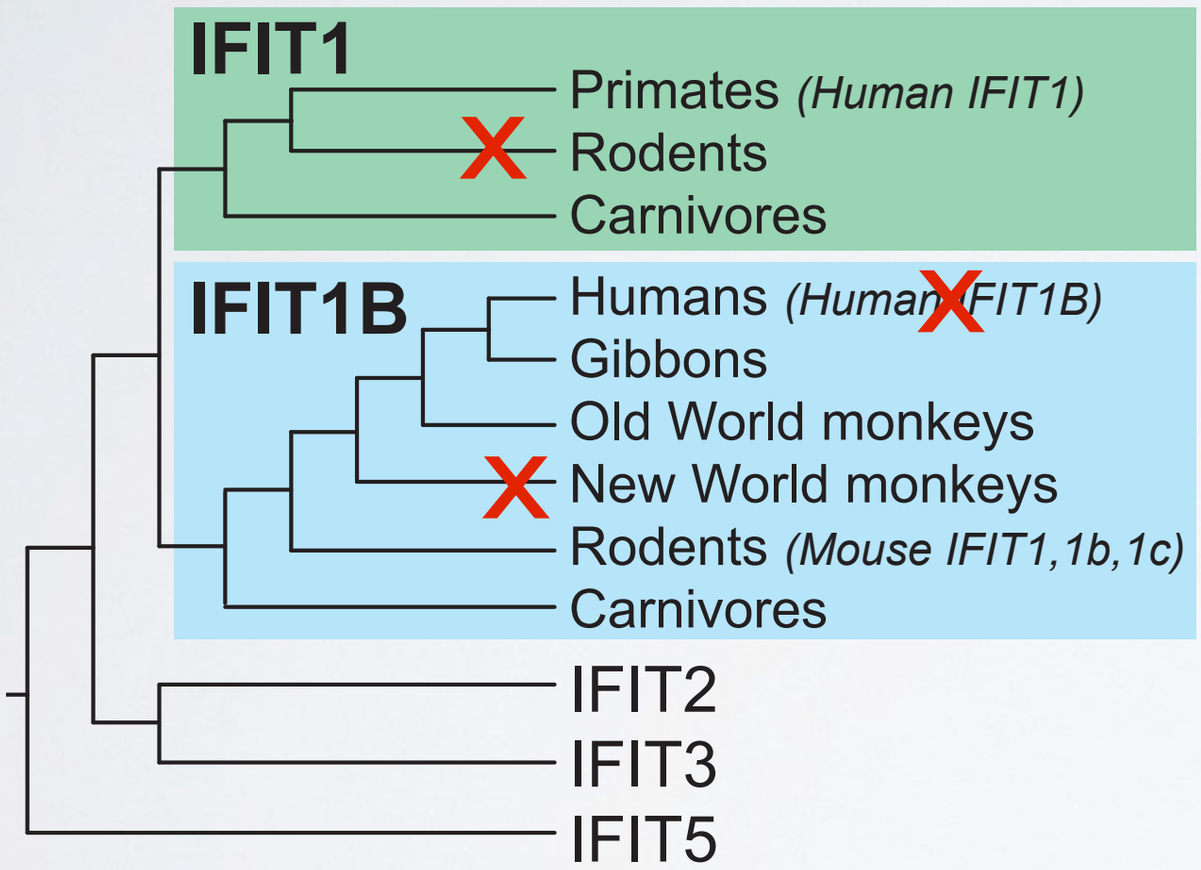


Vaccinia virus





CONSEQUENCES OF IFIT1 EVOLUTION



Antiviral specificity

Molecular recognition

?

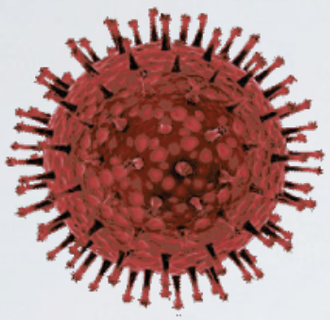
?

Cap0 viruses

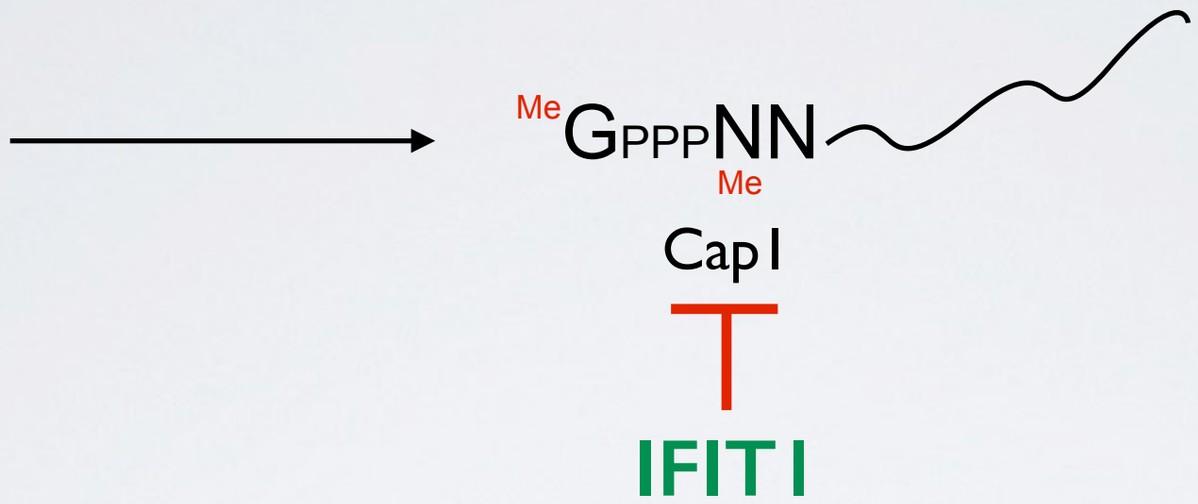
Specific Cap0 recognition

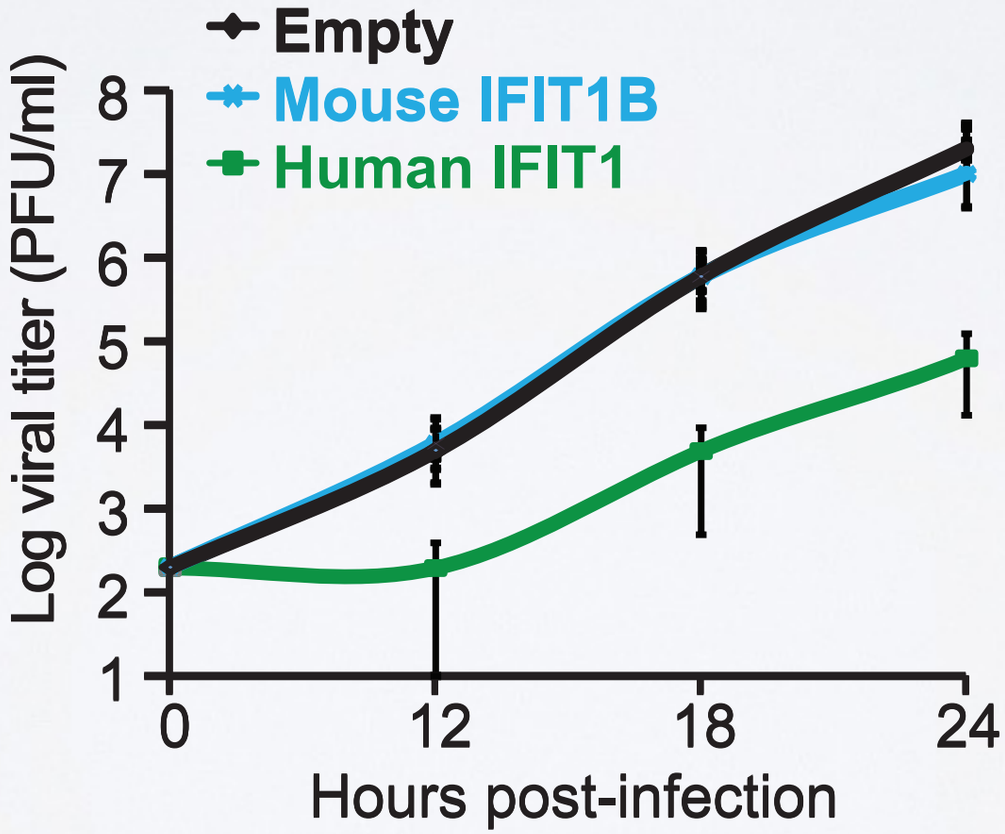
What is the function of IFIT1? Does it have antiviral activity?

IFIT1 INHIBITS CAP1 VIRUS REPLICATION



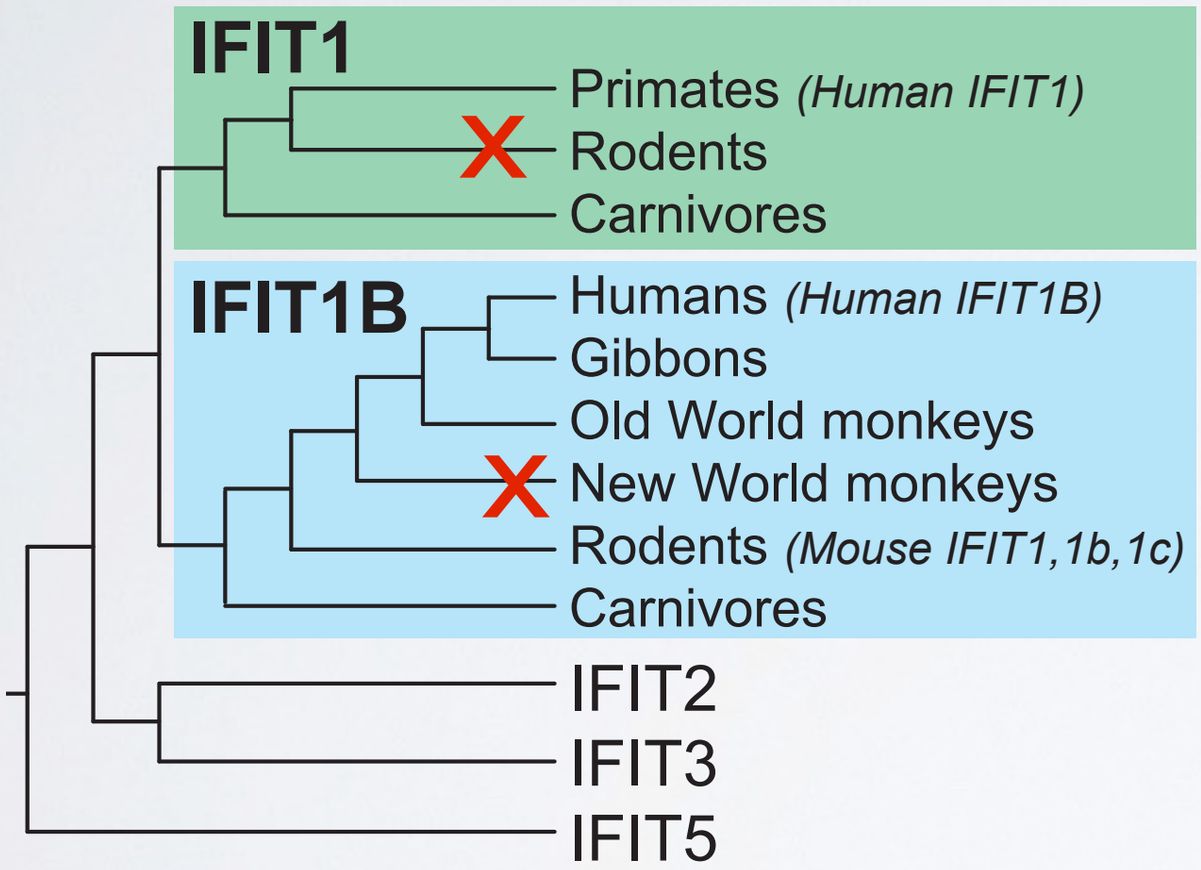
Vesicular stomatitis virus (VSV)

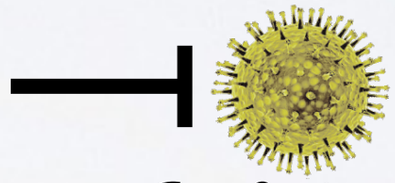




IFIT1 is a potent antiviral, but with specificity that differs from IFIT1B

IFIT1 EVOLUTION ALTERS ANTIVIRAL DEFENSES

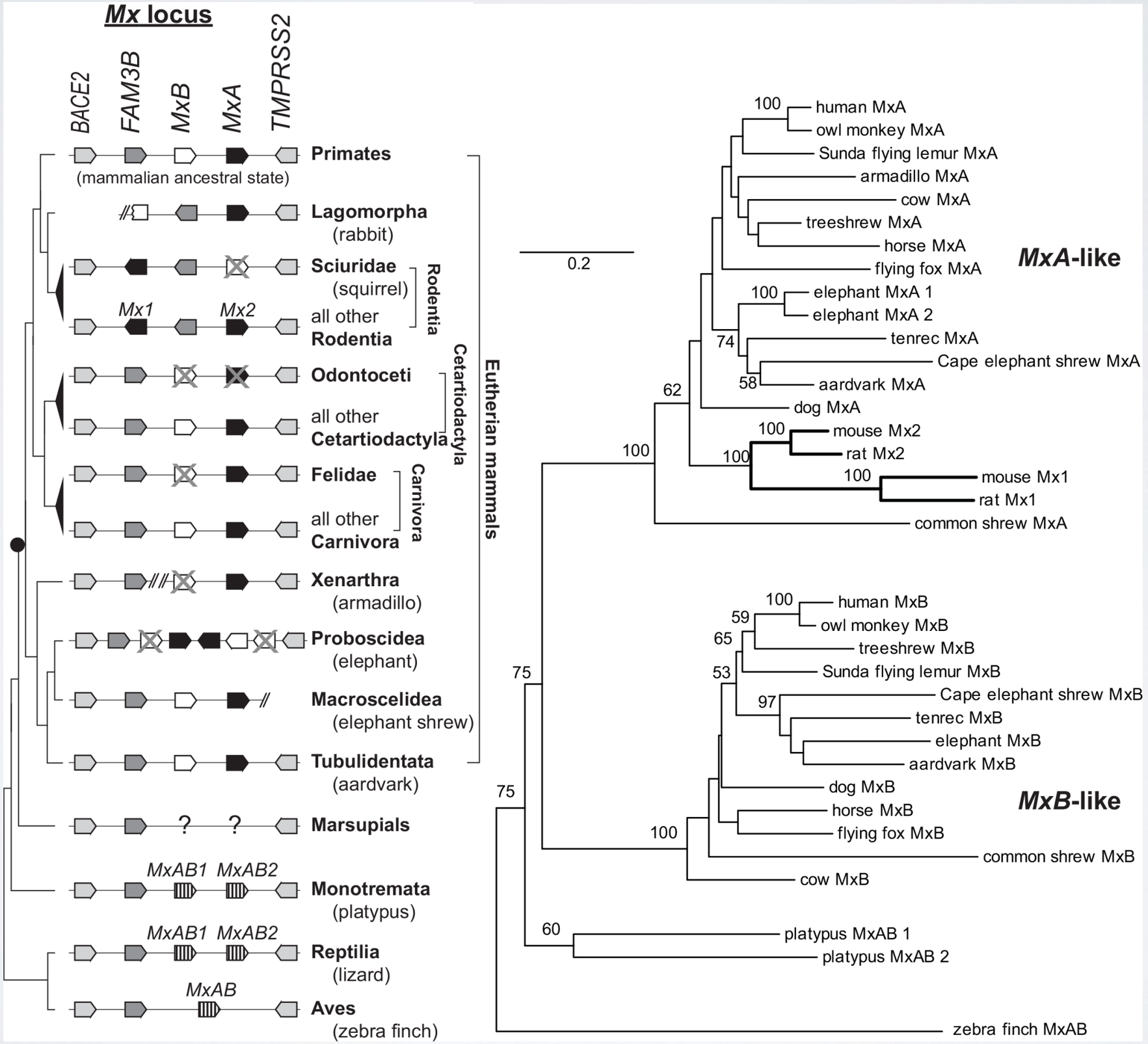


	Antiviral specificity	**Molecular recognition**
IFIT1	 *VSV, others?*	?
IFIT1B	 *Cap0 viruses*	Specific Cap0 recognition

IFIT1 and IFIT1B are distinct gene families that diverged millions of years ago

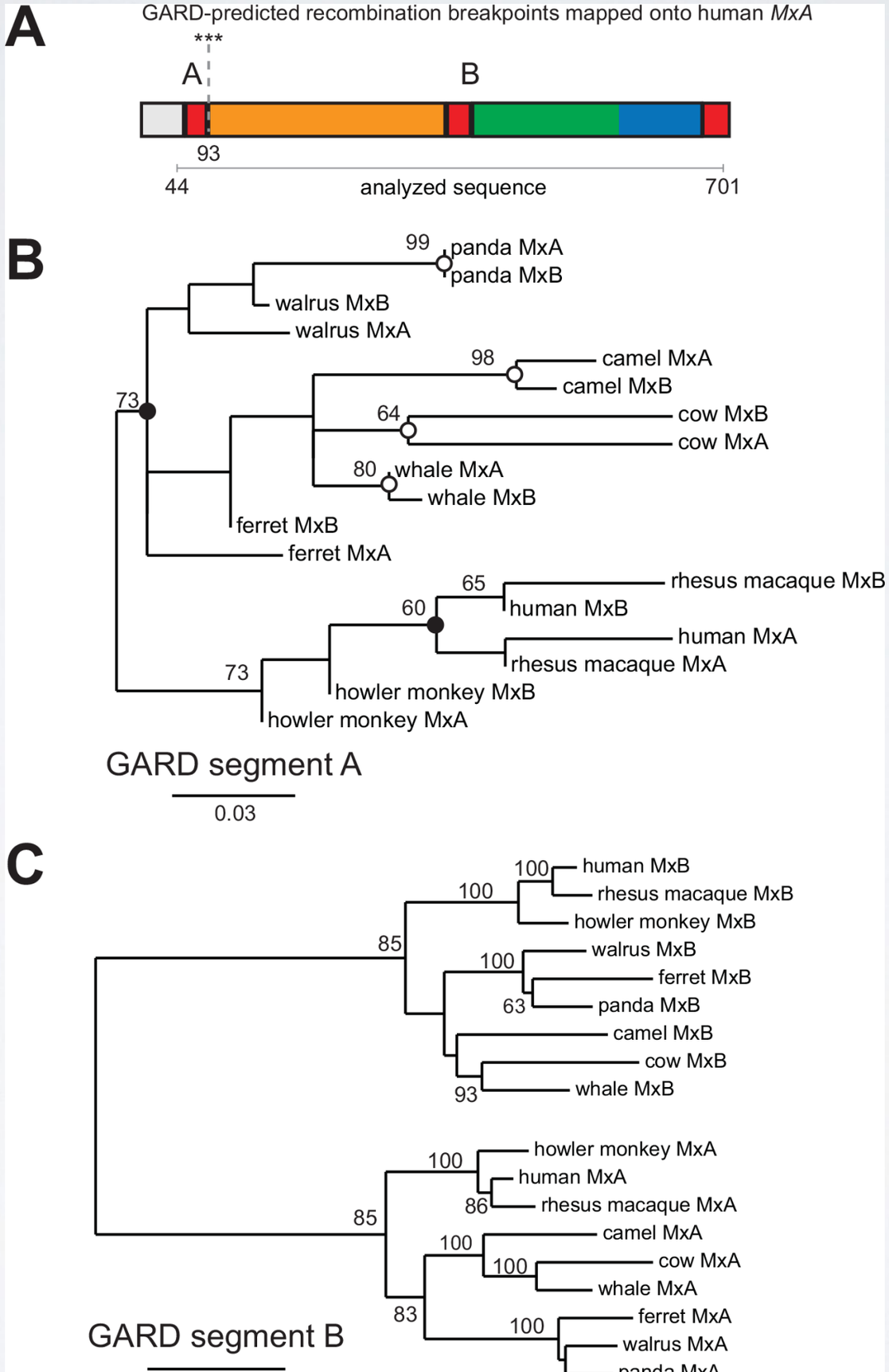
IFIT1 and IFIT1B have evolved different mechanisms and antiviral specificities

Recombination may also help explain MxA/MxB evolution



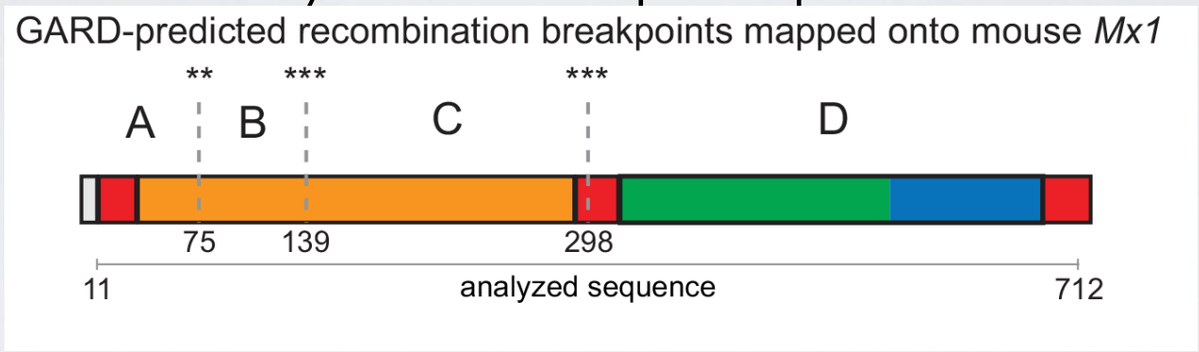
Mitchell et al. PLOS Pathogens 2015

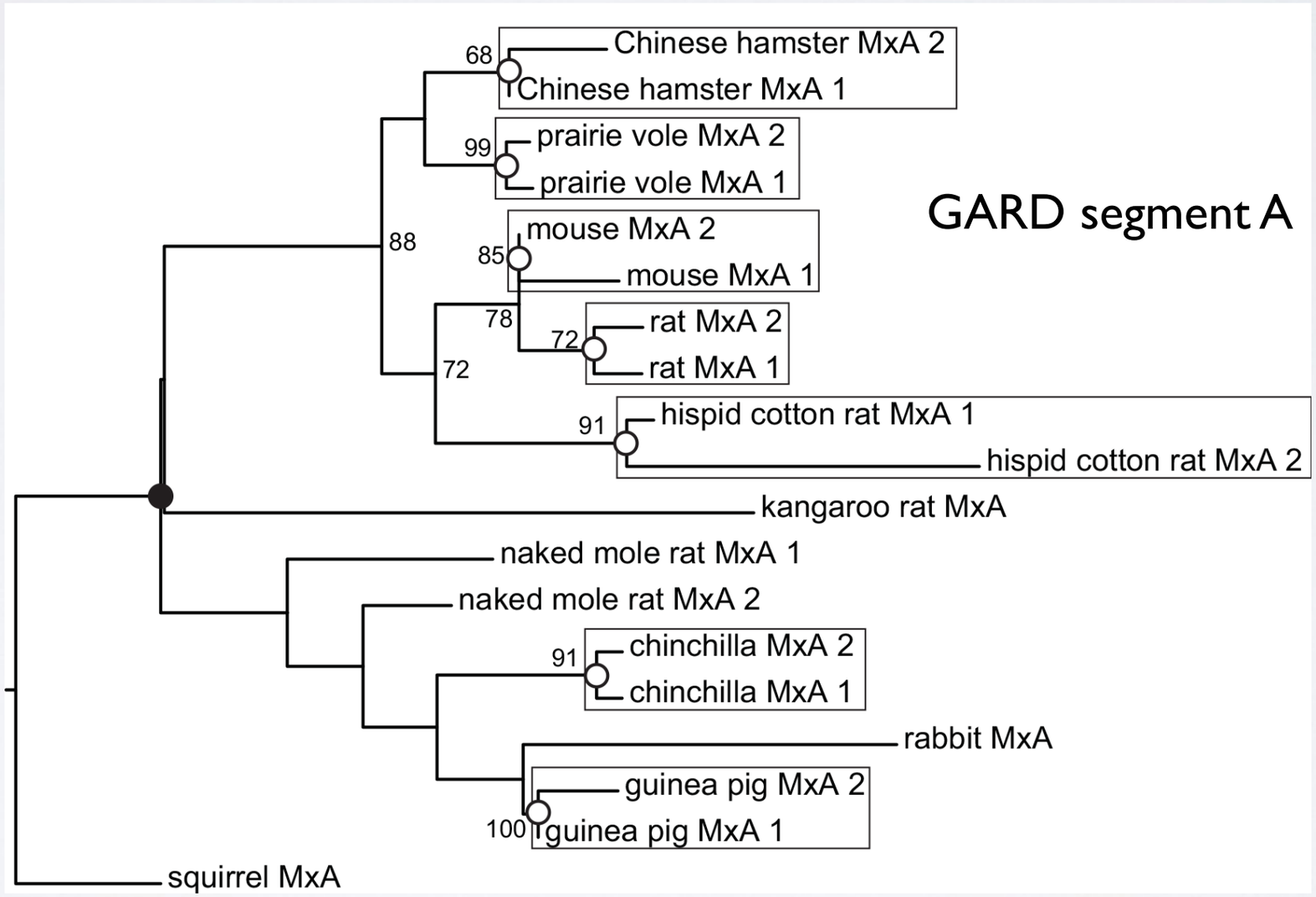
Recombination may also help explain MxA/MxB evolution



Mitchell et al. PLOS Pathogens 2015

Recombination may also help explain MxA/MxB evolution

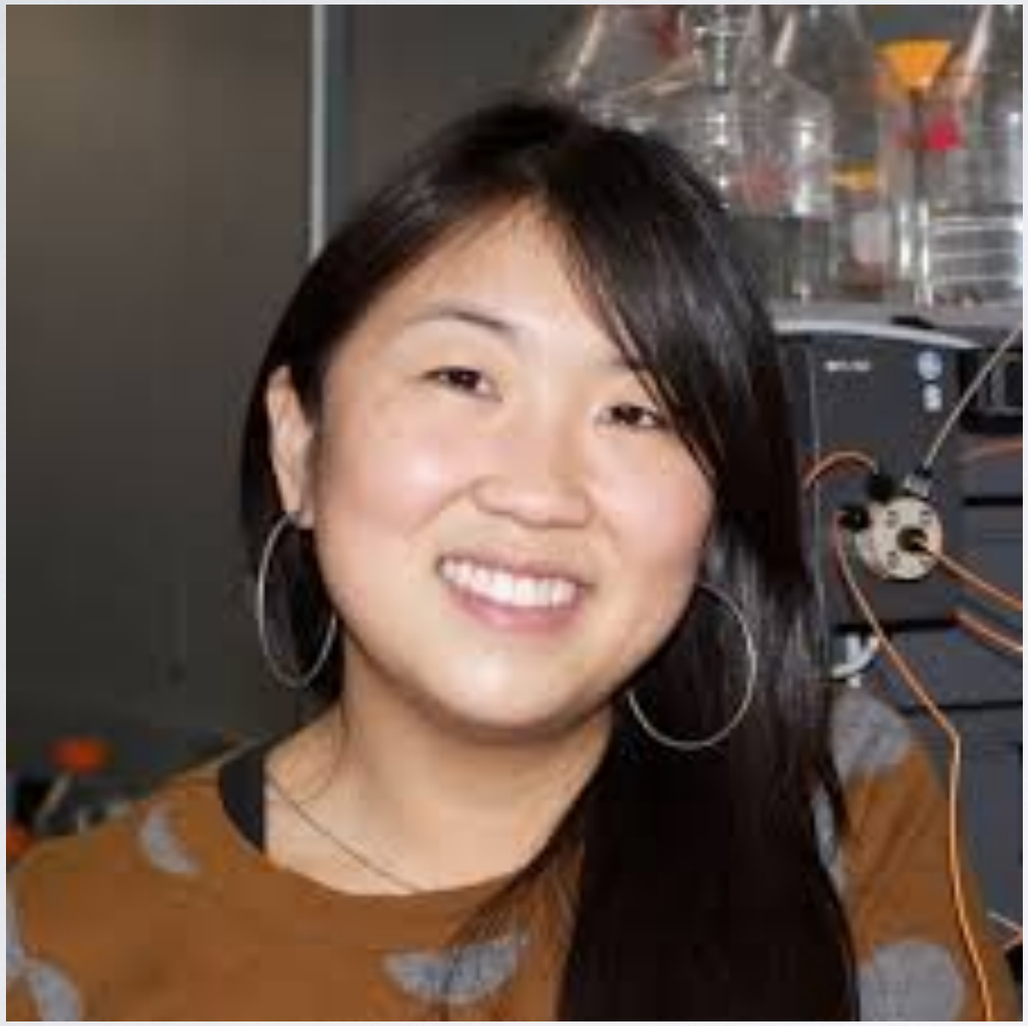




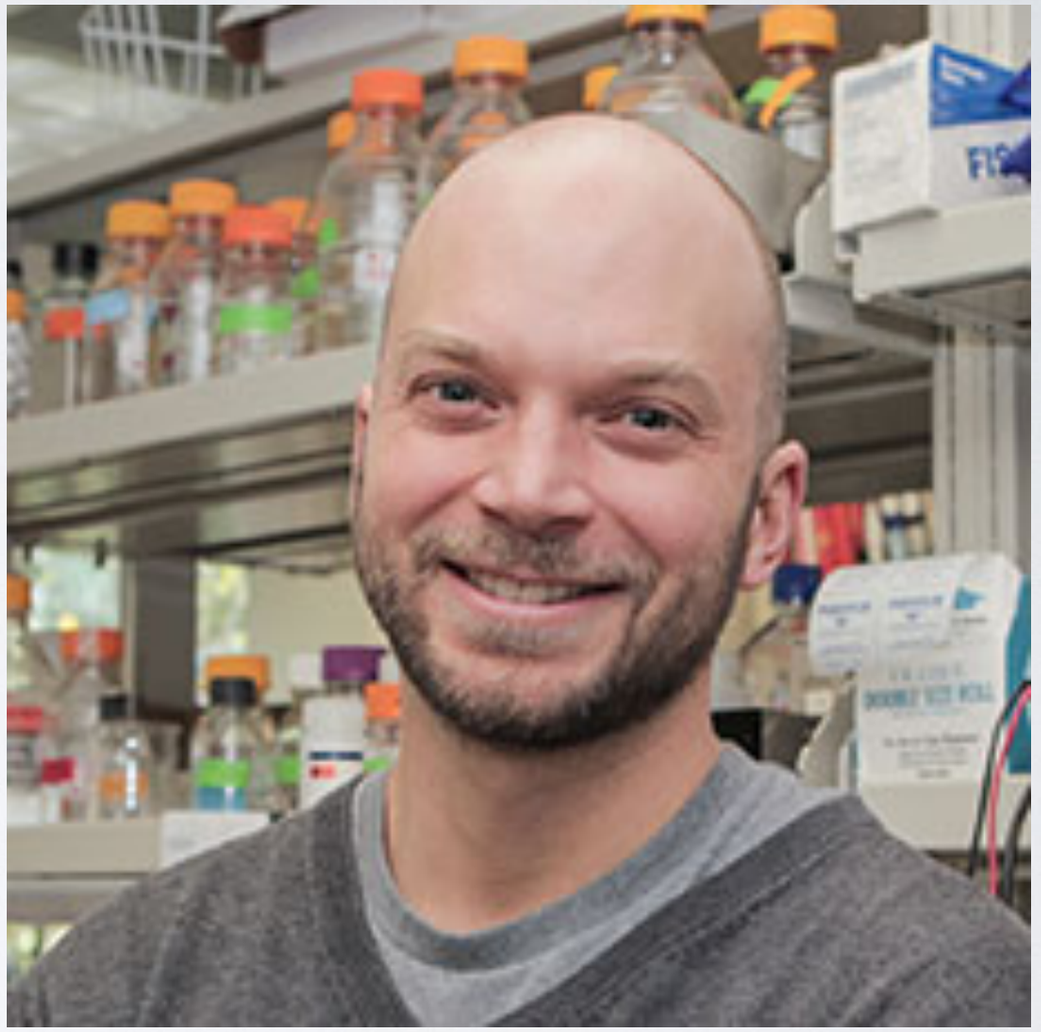
Mitchell et al. PLOS Pathogens 2015

Lecture 3- Vignette 3

An unusual origin for a host immune gene

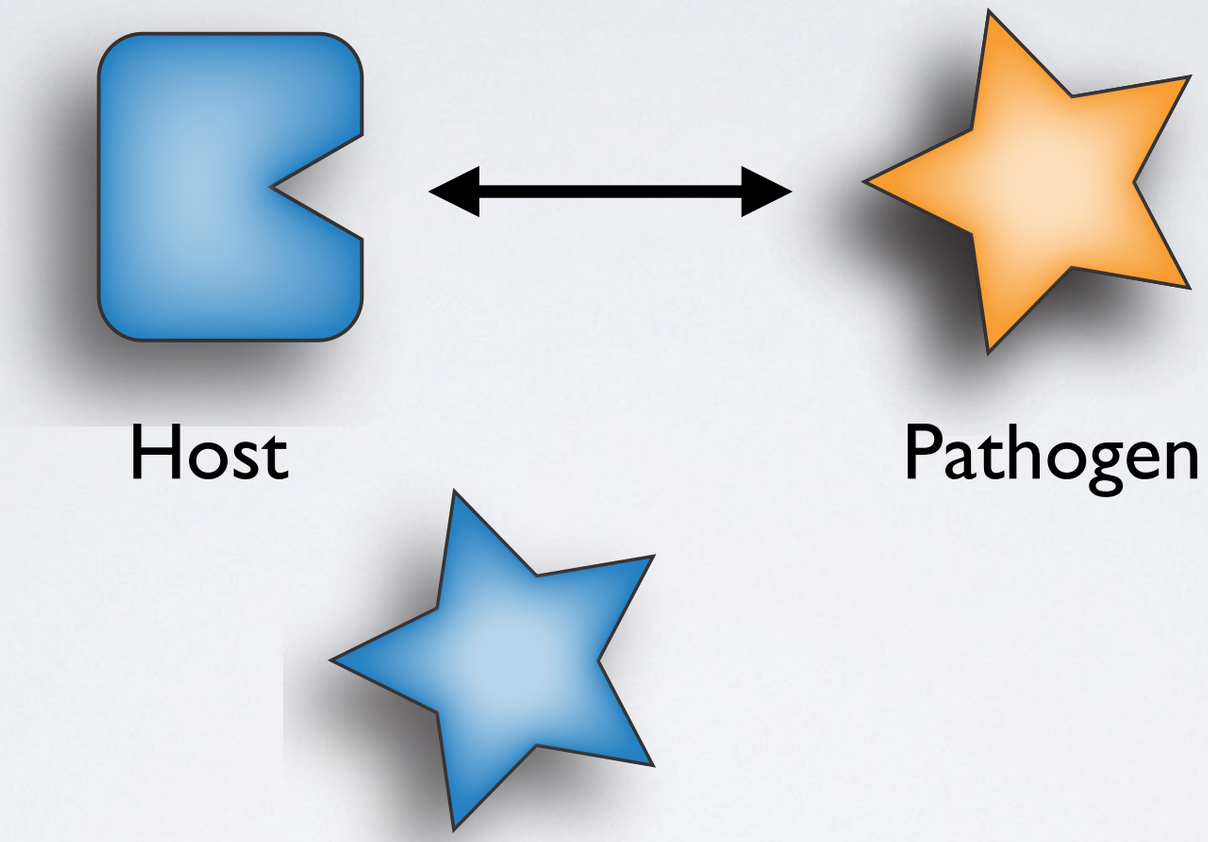


Seemay Chou
former postdoc, Mougous lab, UW
now Assistant Professor, UC San Francisco

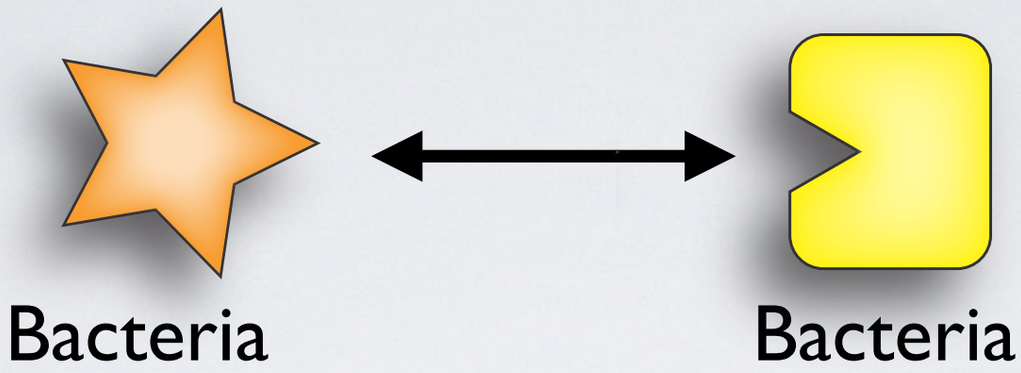


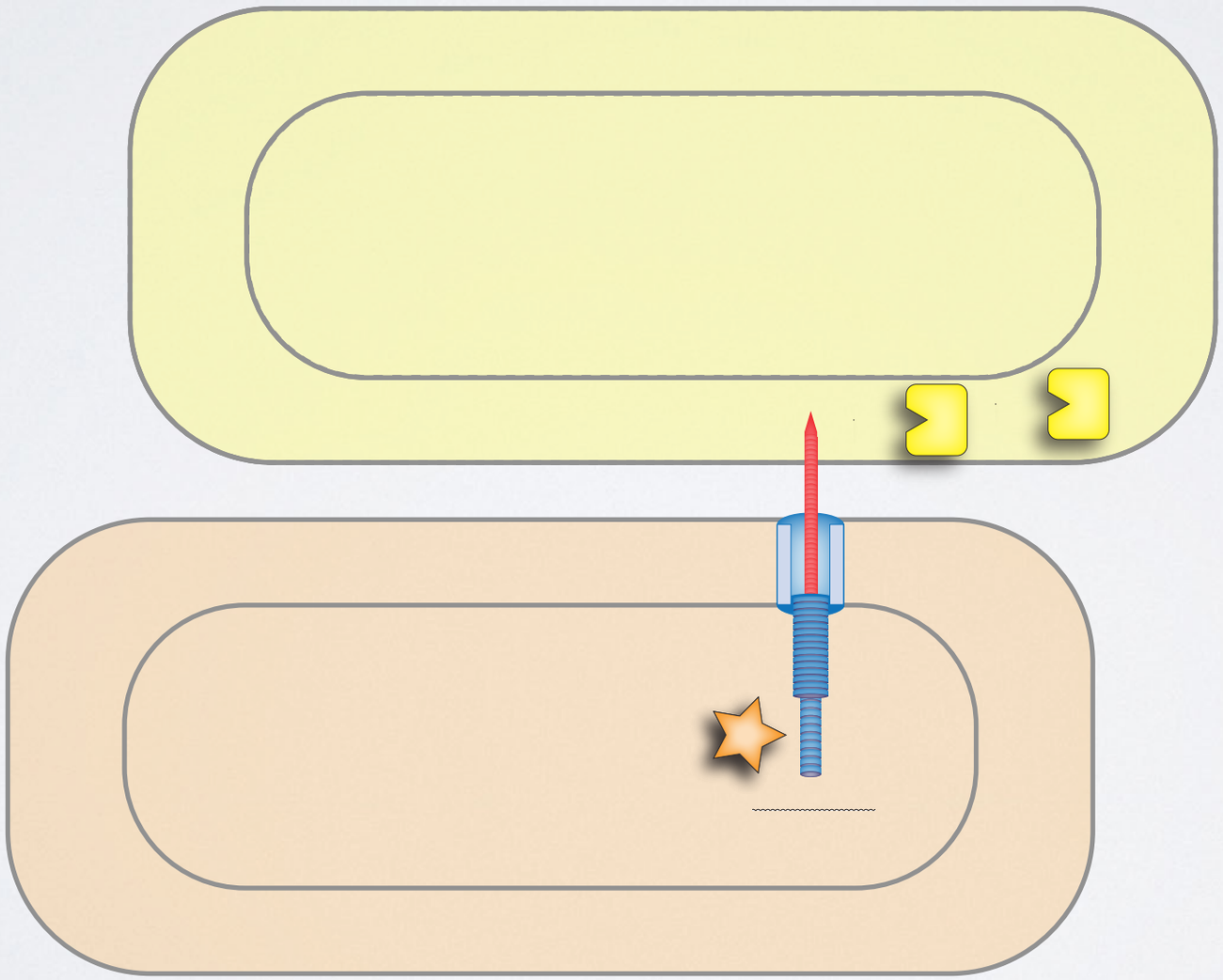
Matt Daugherty, former postdoc
now Assistant Professor, UC San Diego

‘Stealing’ immunity genes

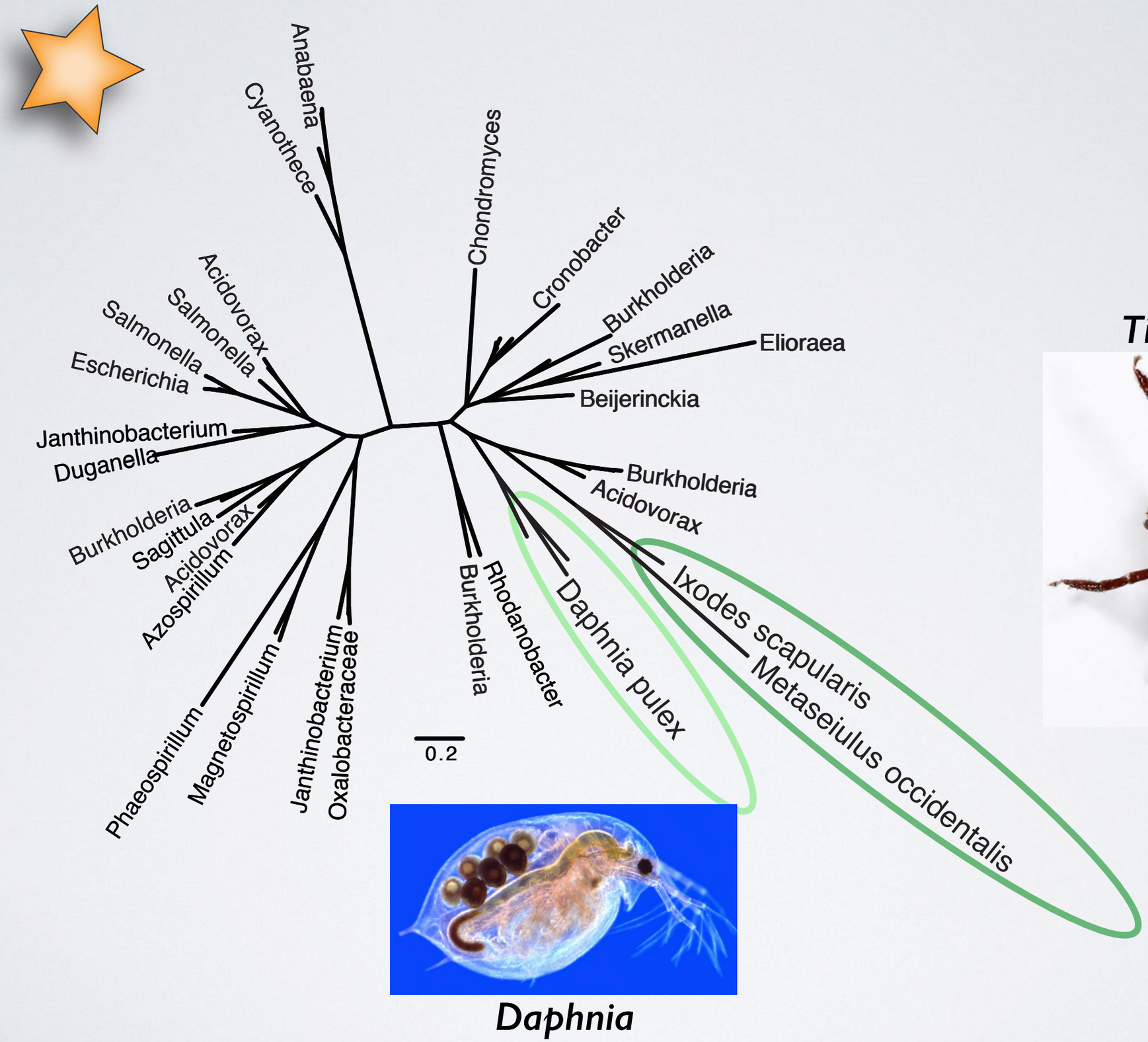


Interbacterial warfare





Bacterial genes in eukaryotic genomes



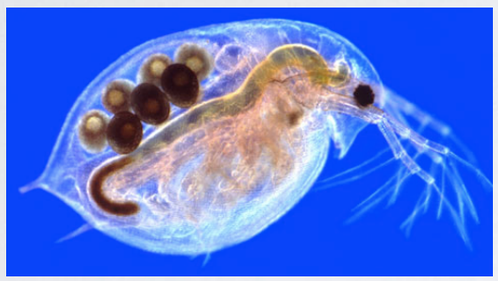
Ticks and mites

Daphnia

Chou, Daugherty*,…Malik, Mougous Nature 2015*

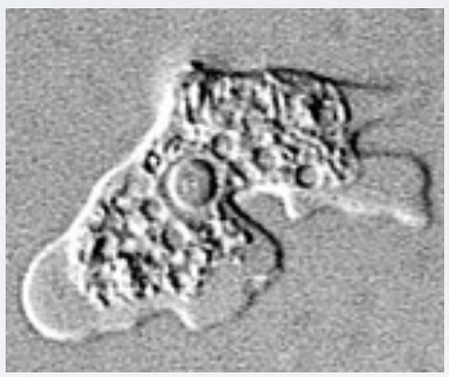
Antibacterial effector genes were ‘stolen’ at least 6 times

Ticks and mites



Daphnia

Oxytricha



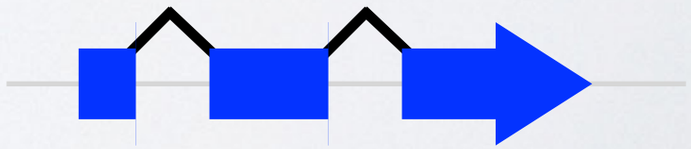
Naegleria

Mollusks

Sea anemone, Lancelet and others

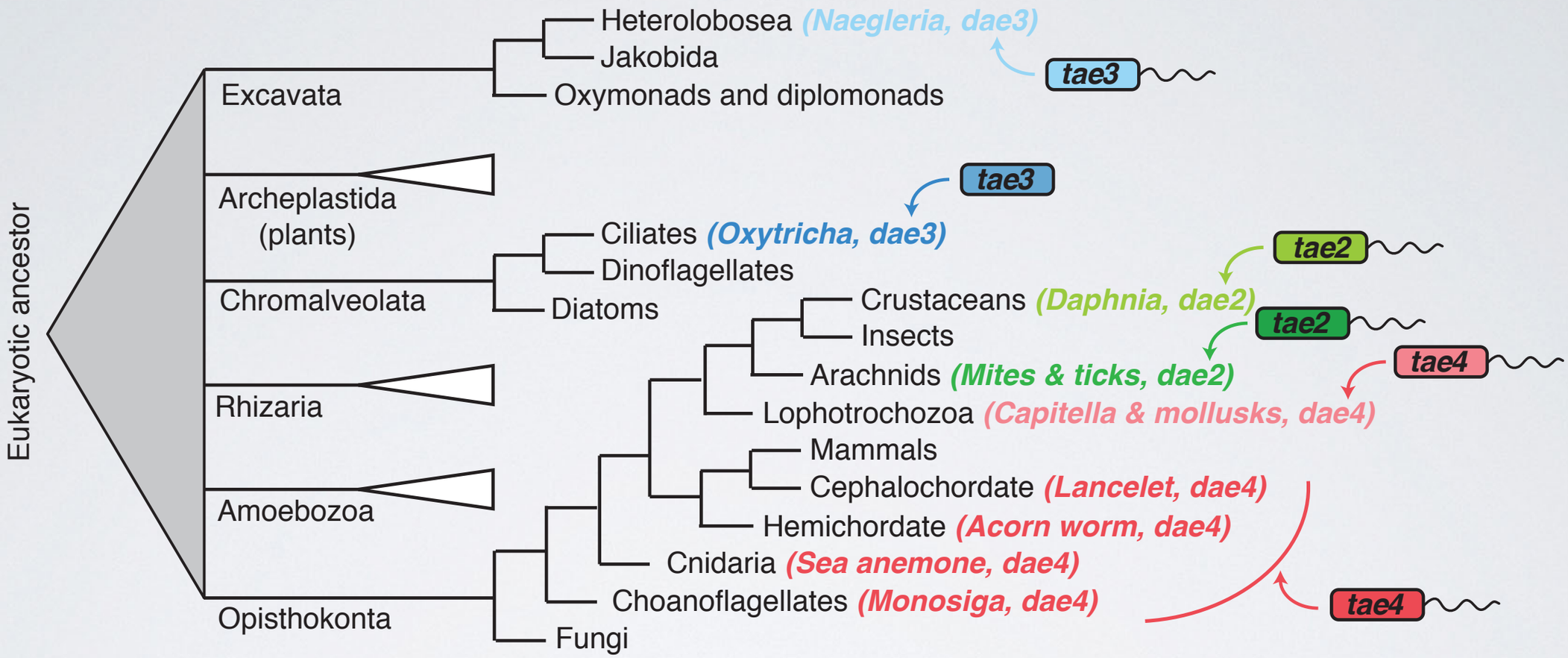
Surrounded by other eukaryotic genes

Contain introns



Chou, Daugherty*,…Malik, Mougous Nature 2015*

GENES WERE 'STOLEN' AT LEAST 6 TIMES

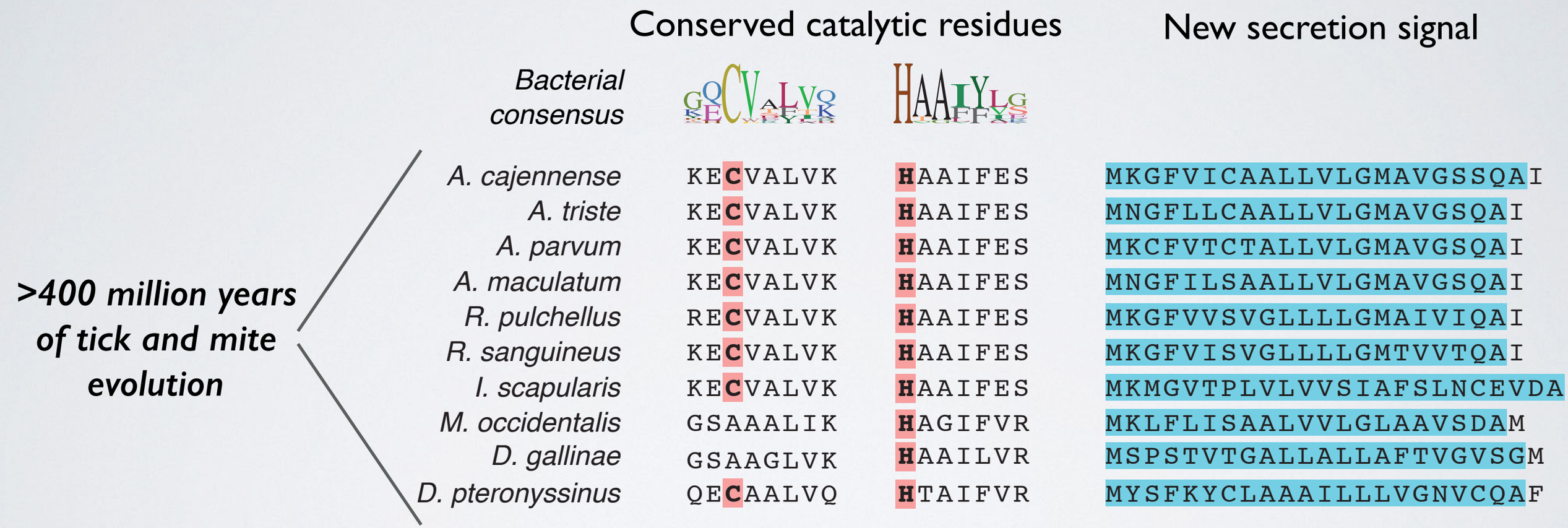


*Renamed **D**omesticated **a**midase **e**ffector (Dae) genes*

Chou, Daugherty*,…Malik, Mougous Nature 2015*

Conservation of catalytic residues

… and a new way to get out of cells

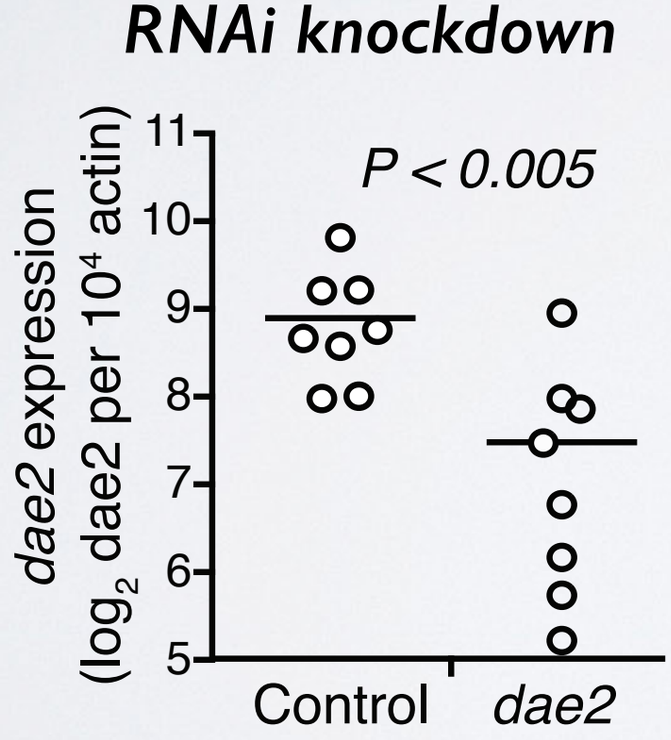


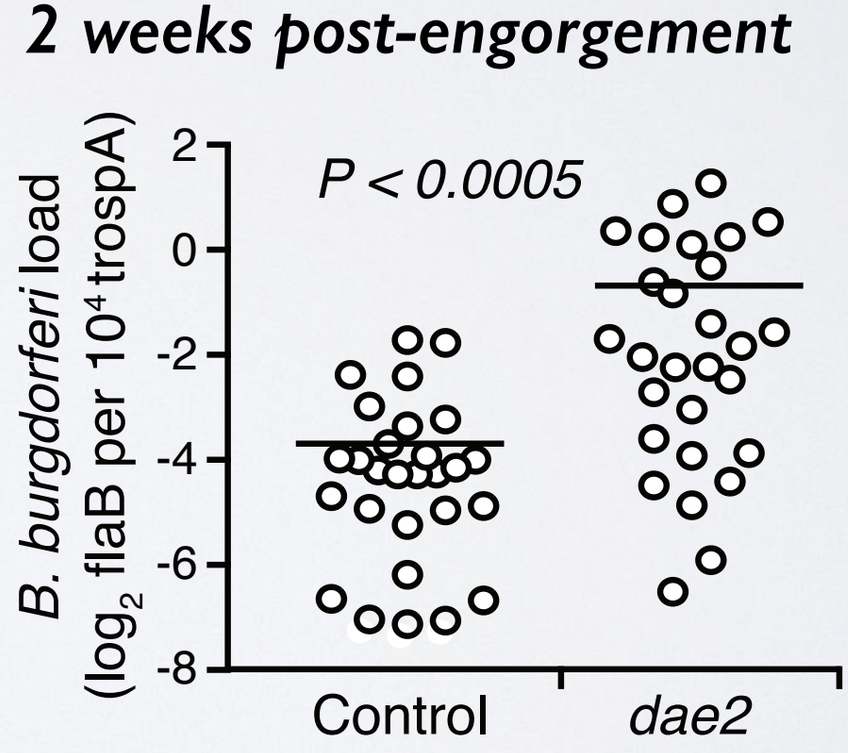
Chou, Daugherty*,…Malik, Mougous Nature 2015*

Dae-mediated antibacterial immunity *in vivo*

Ixodes scapularis (deer tick) is the vector for the Lyme disease pathogen (*Borrelia burgdorferi*)

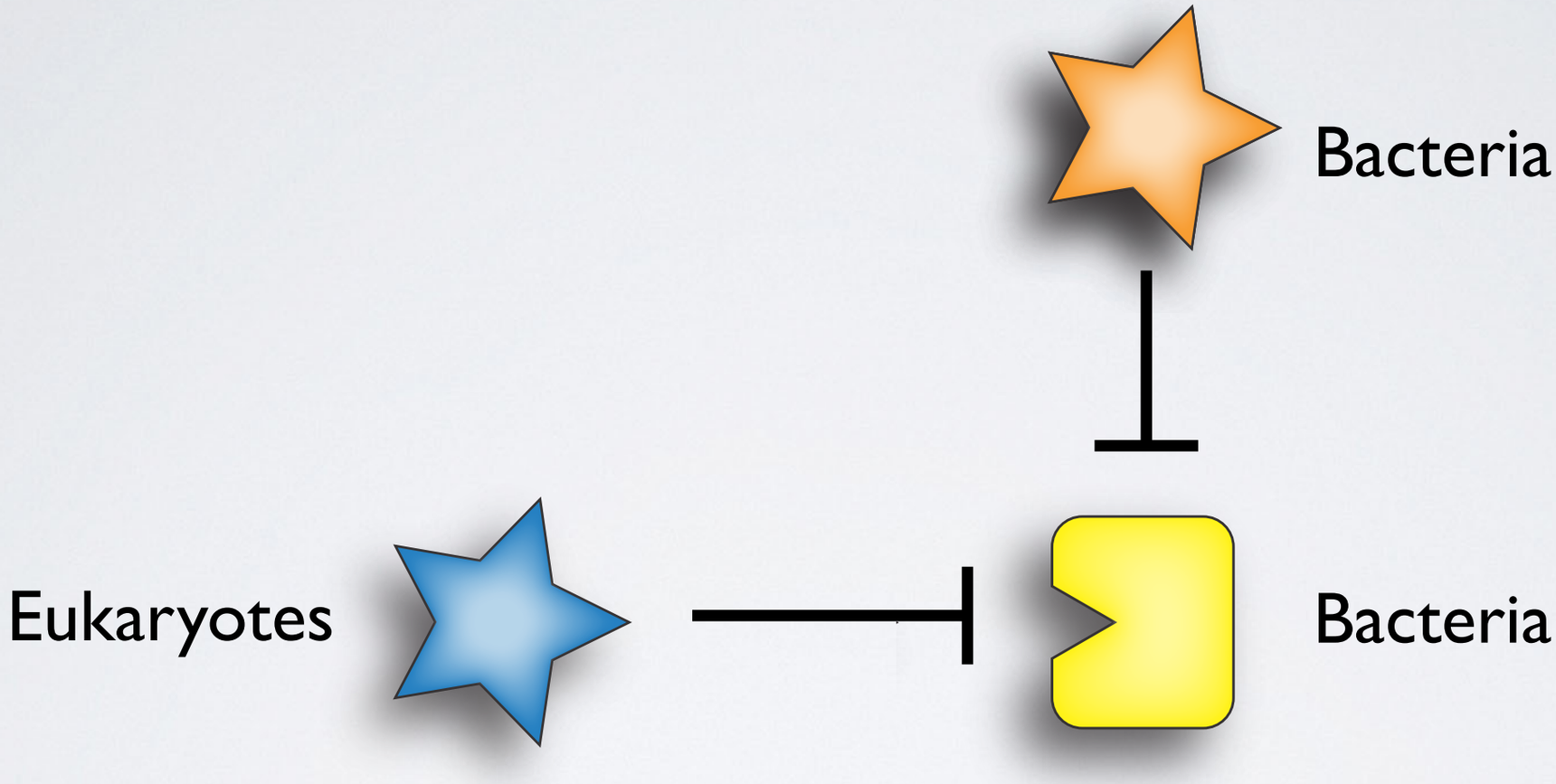
Lyme disease is the most prevalent vector-borne illness in the US





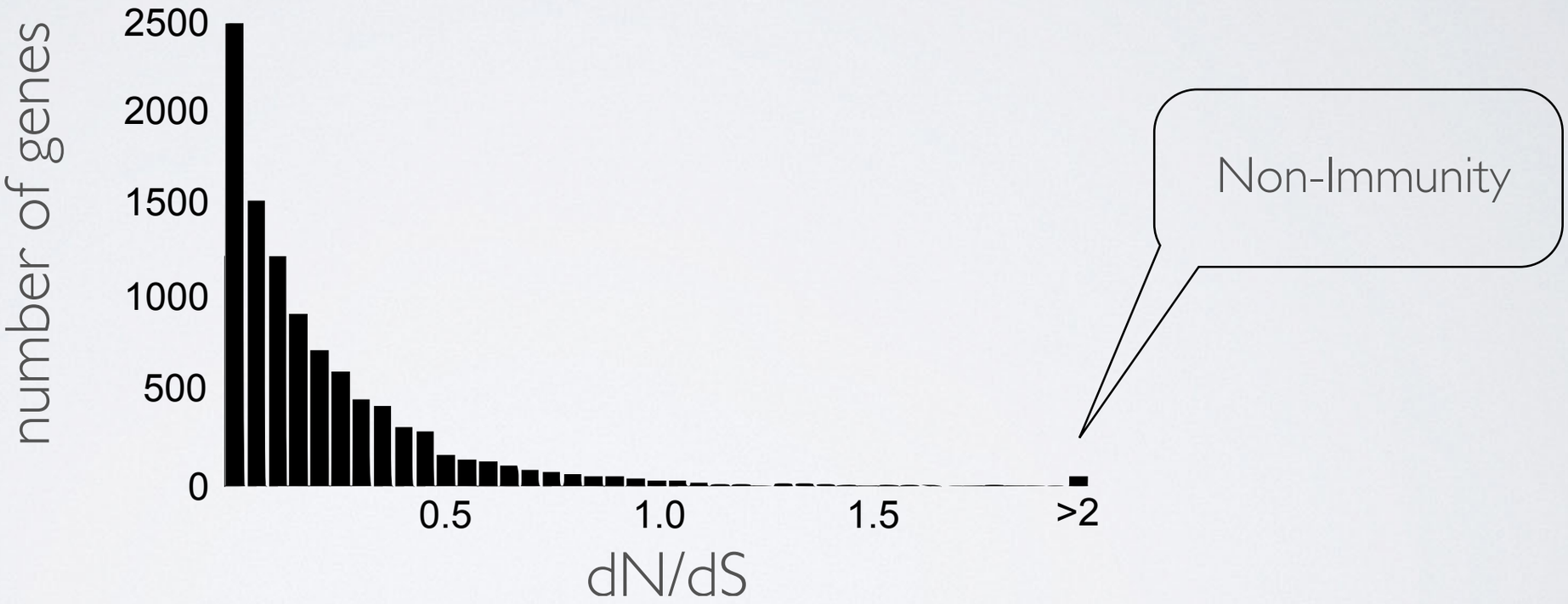
Chou, Daugherty*,…Malik, Mougous Nature 2015*

Horizontal gene transfer as a mechanism to acquire immunity



By stealing well-honed interbacterial toxins, eukaryotes get 'out-of-the-box' antibacterial immunity factors

GENOMIC VIEW OF SELECTION

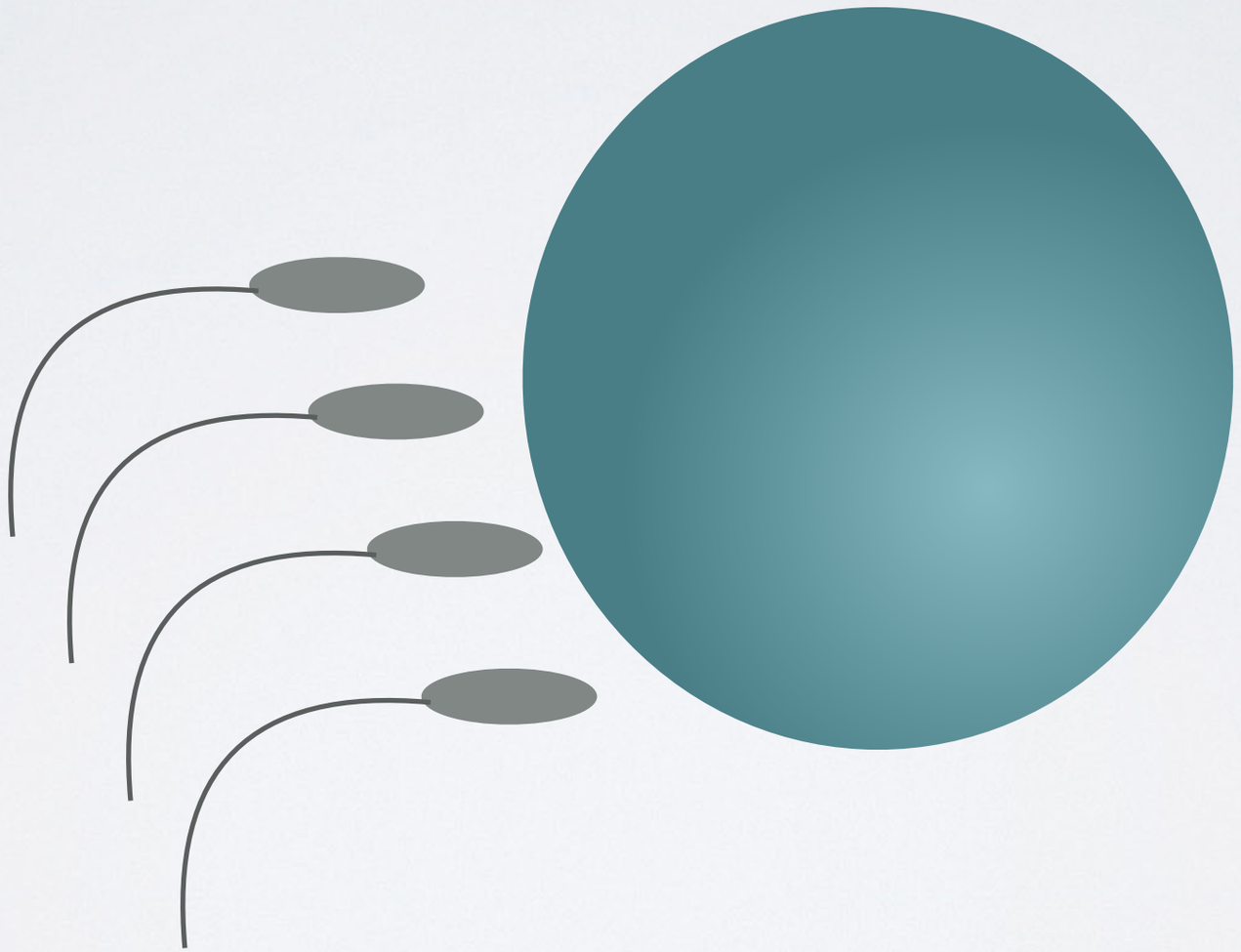


purifying —— neutral — positive selection

Siepel Lab

beyond the usual suspects

Genetic conflicts between components of
the same cell and even same genome



beyond the usual suspects

Genetic conflicts between components of
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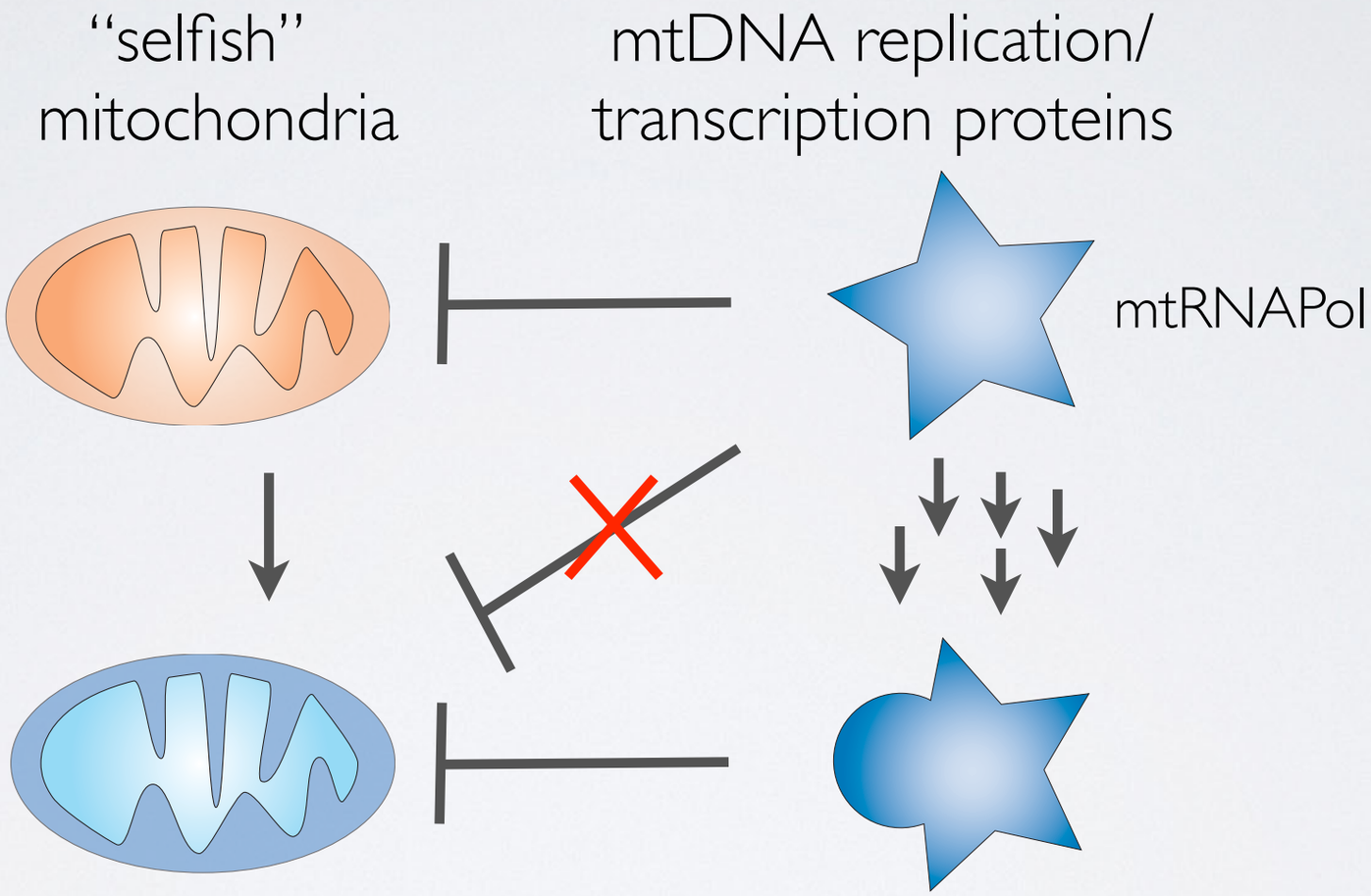
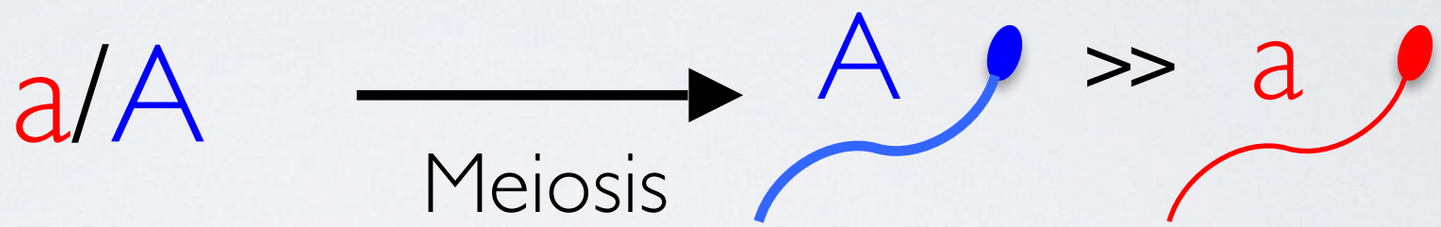


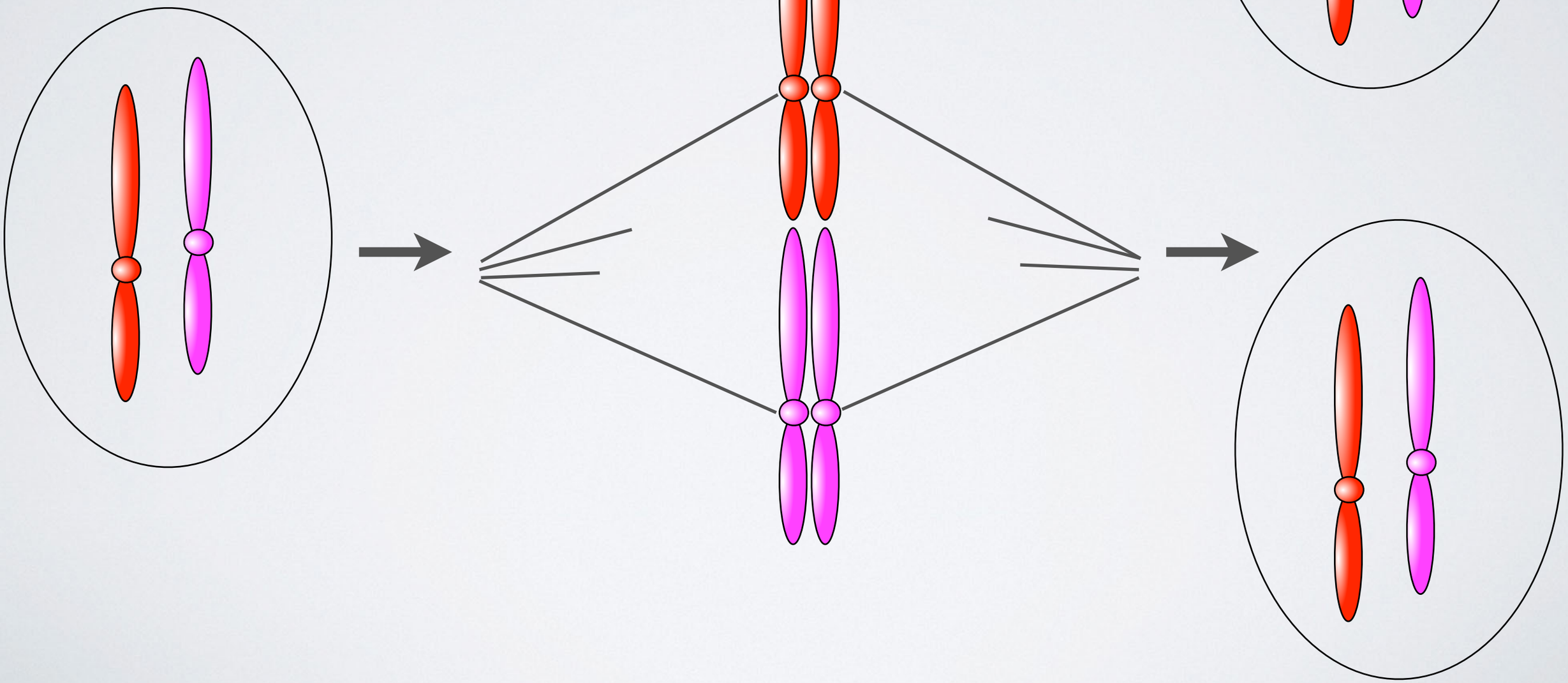
beyond the usual suspects

Genetic conflicts between components of the same cell and even same genome



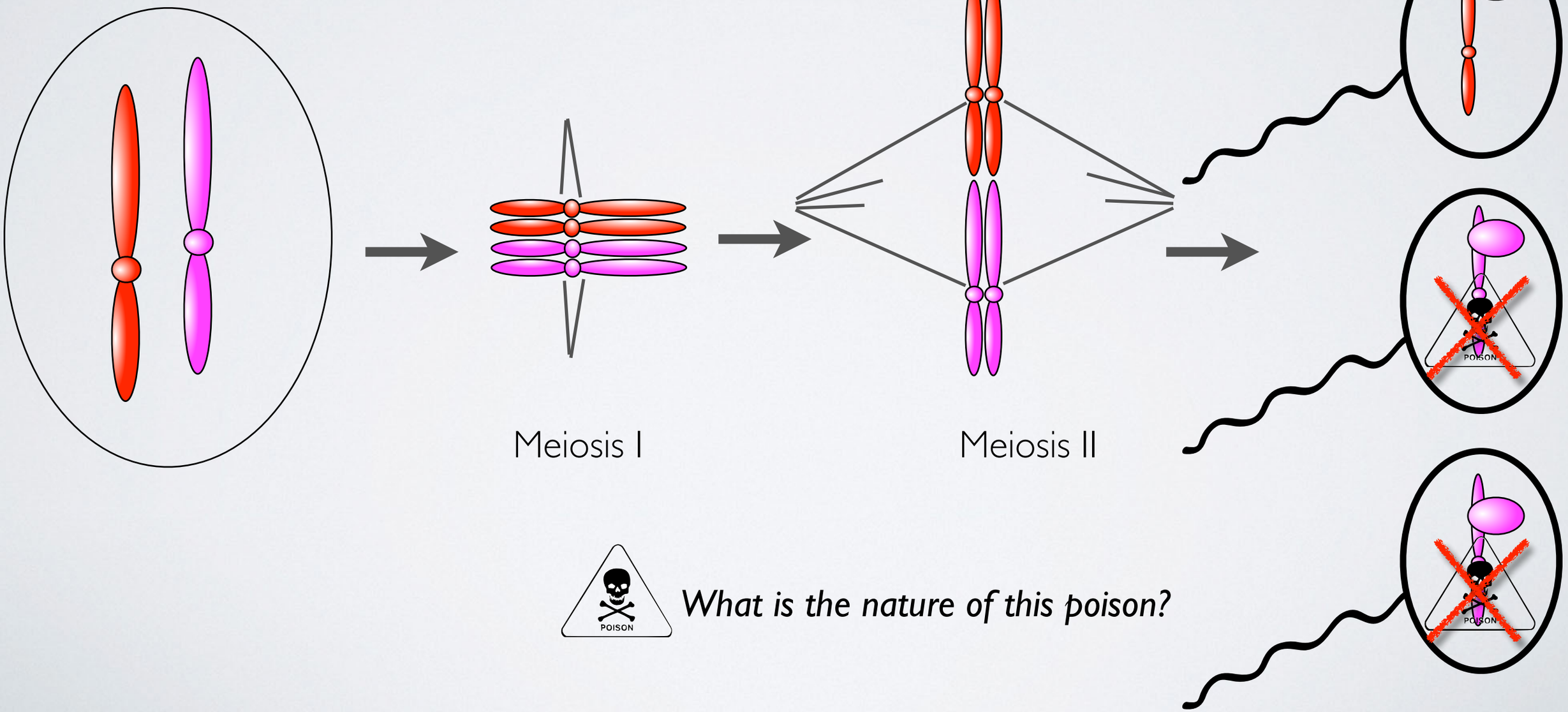
- Alleles are evolutionary competitors
  - fight fair (promote fitness; fitter allele ‘wins’)
  - cheat to win during/after meiosis; unfit allele can sometimes ‘win’

Mitosis



‘Cheating’ **after** chromosome segregation

Male meiosis

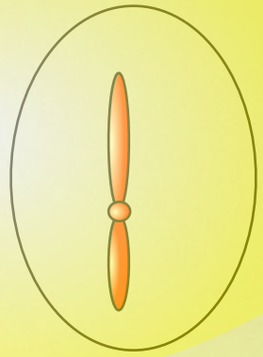


What are possible suppressors?

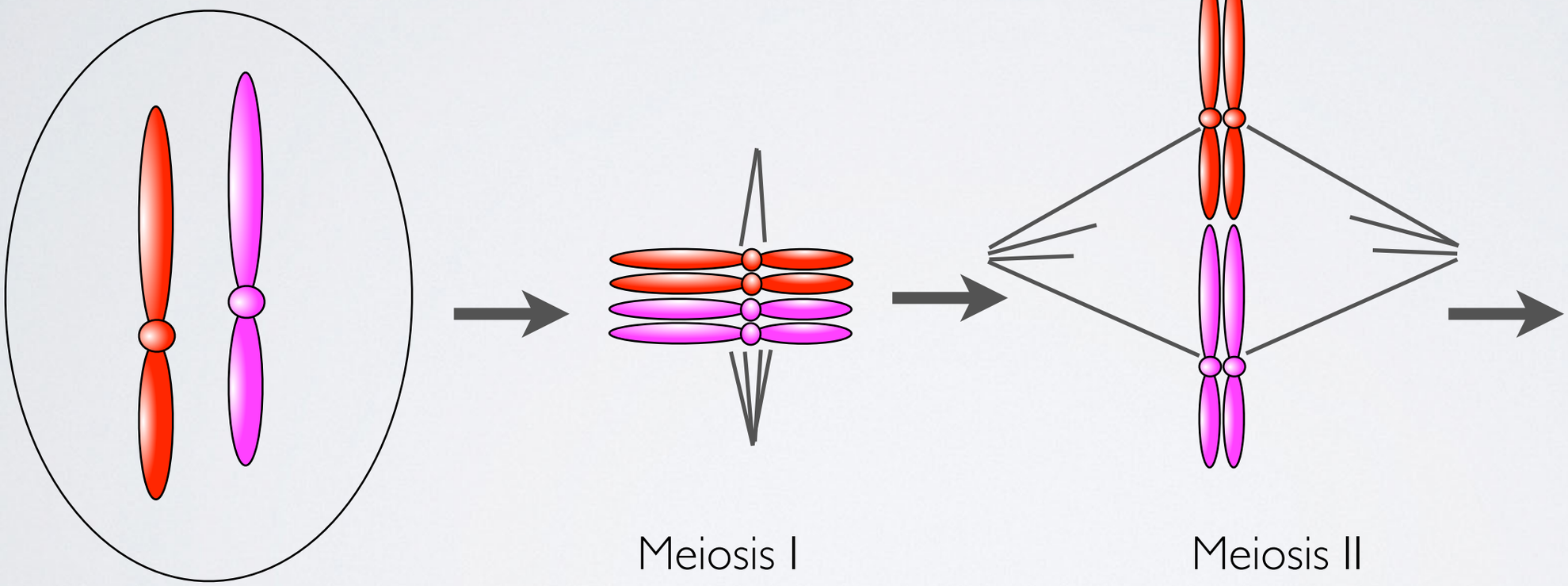
What is the nature of this poison?

‘Cheating’ **during** chromosome segregation

oocyte
oocyte



Female meiosis



Meiosis I

Meiosis II