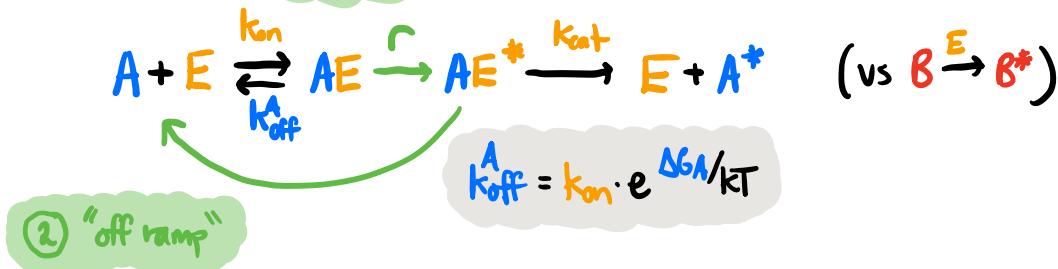


Last time: molecular errors & kinetic proofreading

Simple example: ① Irreversible step (e.g. ATP)



$$\Rightarrow \frac{\text{rate of error product}}{\text{rate of correct product}} = \frac{[B]}{[A]} \left(e^{\frac{\Delta G_A - \Delta G_B}{kT}} \right)^2$$

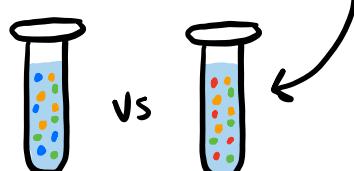
irreversible step buys time to "test" k_{off} twice!

Tradeoffs: ① "wastes" energy ② slower / lower throughput

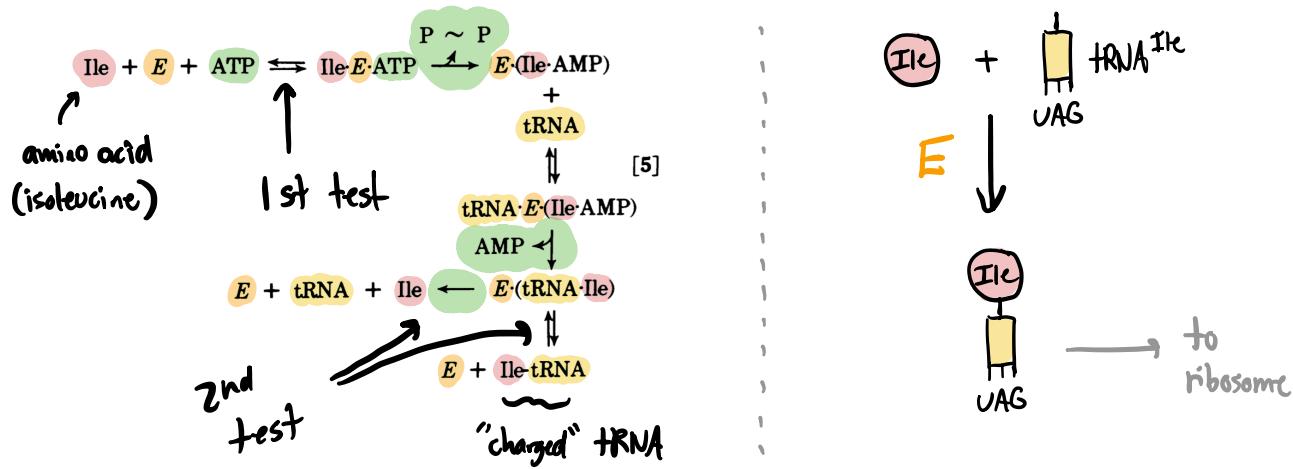
\Rightarrow Hopfield et al '76: test for kinetic proofreading

$$\frac{\text{rate of energy use}}{\text{rate of product}} = 1 + \frac{k_{off}}{k_{cat}}$$

→ Should be larger for errors than for correct product!



Hopfield et al '76: applications to tRNA charging



In vitro measurements:
 Ile (correct) : ~1.5 ATP per product
 Val (error!) : ~270 ATP "

⇒ error is ~270x more likely to dissociate than form product
 (vs 1.5x for correct amino acid ⇒ ~30% "wasted")

⇒ decreases error rate by extra factor of $\frac{270}{1.5} = 180$

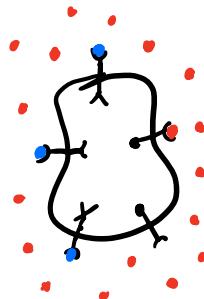
⇒ initial error rate ($Ile + E + ATP \rightleftharpoons Ile-E \cdot ATP$) $\sim 1/100$

⇒ total error rate =

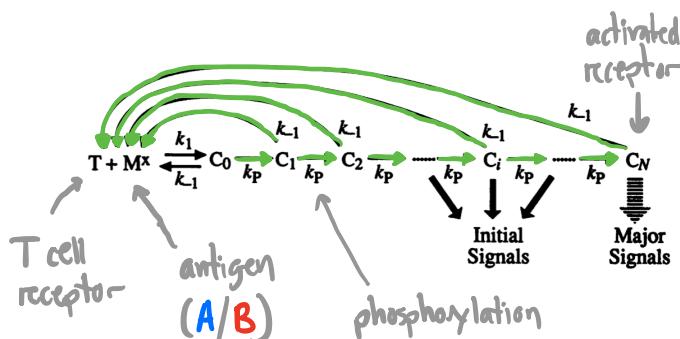
⇒ general idea can be extended to many other contexts

E.g. T-cell receptor Signaling in immune system

Problem: how to detect low conc. of **foreign antigens** in sea of weakly binding **self-antigens**?



⇒ McKeithan '95: proofreading via multiple phosphorylation steps?

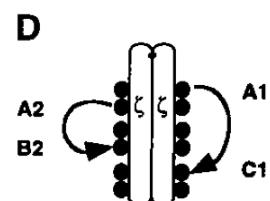


$$\frac{C_N^B}{C_N^A} = \frac{[B]}{[A]} \left(\frac{k_p + k_A^N}{k_p + k_B^N} \right)^{N+1}$$

Fidelity of T Cell Activation Through Multistep T Cell Receptor ζ Phosphorylation

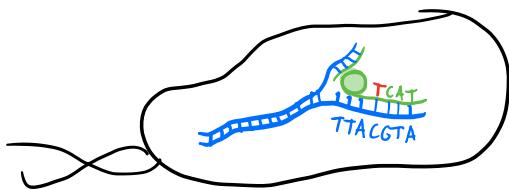
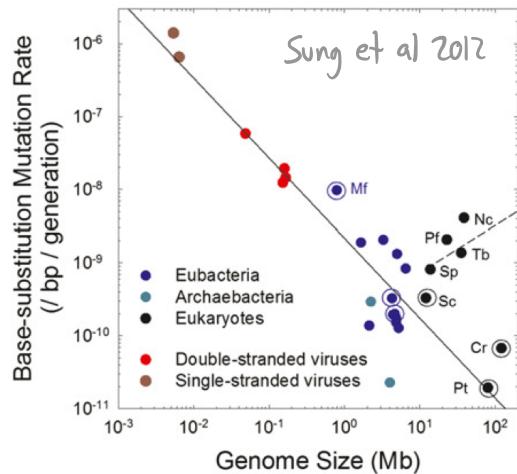
Ellen Neumeister Kersh, Andrey S. Shaw, Paul M. Allen*

The T cell receptor (TCR) $\alpha\beta$ heterodimer interacts with its ligands with high specificity, but surprisingly low affinity. The role of the ζ component of the murine TCR in contributing to the fidelity of antigen recognition was examined. With sequence-specific phosphotyrosine antibodies, it was found that ζ undergoes a series of ordered phosphorylation events upon TCR engagement. Completion of phosphorylation steps is dependent on the nature of the TCR ligand. Thus, the phosphorylation steps establish thresholds for T cell activation.



Phosphorylation sites on ζ component of TCR

⇒ Started w/ discussion of errors during DNA replication...



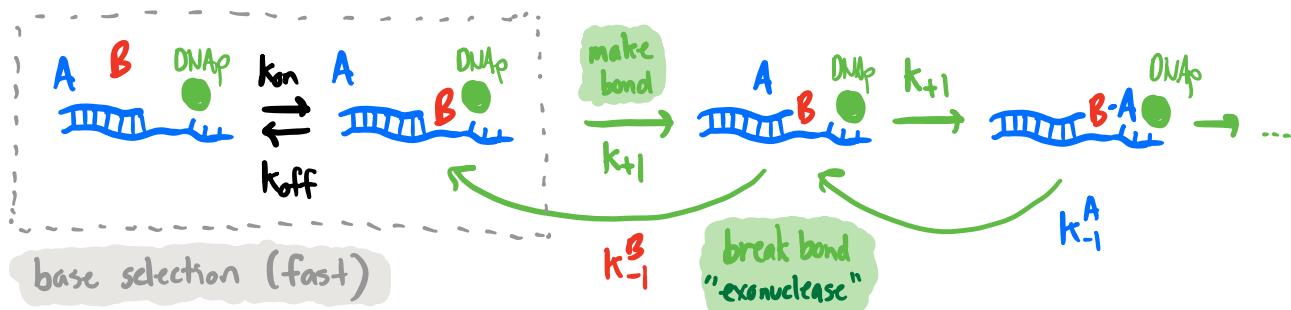
⇒ what do real cells do?

⇒ a little more complicated than above...

⇒ some key factors:

① DNAP can modify dNPs relative to value in sol'n (e.g. exclude H_2O)
(see e.g. Petruska et al '86)

② Many DNAPs also use "exonuclease proofreading"

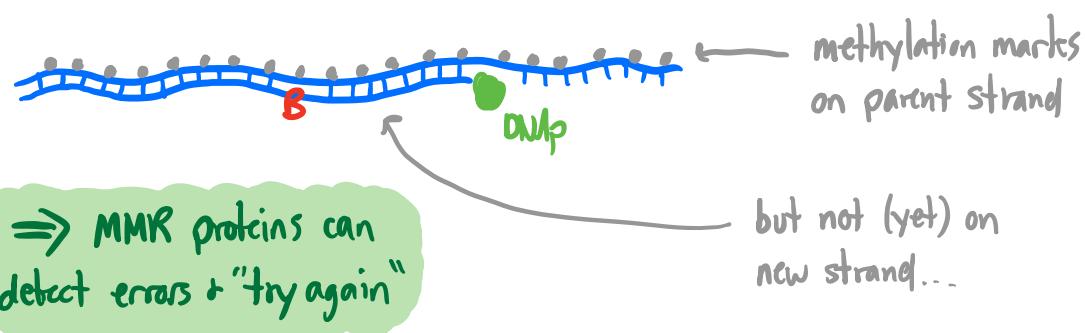


⇒ not technically kinetic proofreading, but similar idea:

- (i) Irreversible step + "off ramp" can enhance specificity
- (ii) Tradeoffs = "wasted" energy + reduced processivity

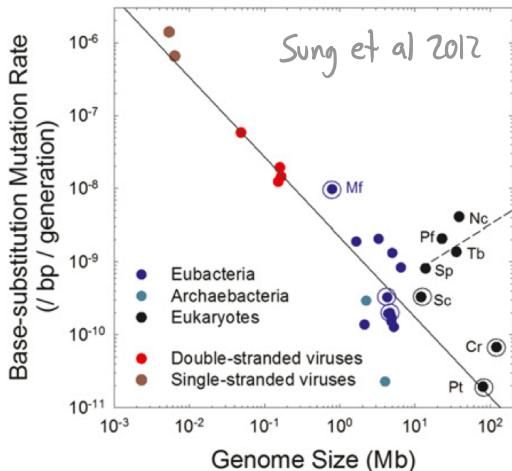
③ Additional error correction mechanisms beyond DNAP

E.g. mismatch repair (MMR) pathway



knocking out these genes can increase mutation rates ~ 10-1000x

Upshot: cells have evolved multiple layers of machinery to reduce mutation rates below $\exp(-\Delta G_0/kT)$



mut'n rate is under
genetic control &
can evolve over time!

Question: why these values? could evolution do "better"?

⇒ will see that physics (diffusion!) imposes fundamental limits on evolution as well...

⇒ systematic treatment in:

APPHYS 237/BIO 251:
"Quantitative evolutionary dynamics & genomics"

⇒ will outline basic flavor here...

Question: why would evolution select for lower mut'n rates?

\Rightarrow one possibility: fewer lethal mutations

e.g. E. coli genome: 
"essential" sites
(mutation = death)

\Rightarrow probability of viable offspring = $1 - \mu L p_x$

μ = mutation rate / bp/division ($\sim 10^{-10}$ in E.coli)

L = length of genome ($\sim 4 \times 10^6$ in E.coli)

p_x = prob. that mut'n is "lethal" ()

\Rightarrow Upper bound on mut'n rates: $\mu < 1/L p_x$

\Rightarrow but observed values much lower...

key insight: rare lethal mut'n's still impose weak fitness costs that can add up over time...

E.g. E.coli w/ "super MMR" pathway ($\mu \rightarrow 0$)



$$\Pr[\text{viable offspring}] = 1$$



$$\Pr[\text{viable offspring}] = 1 - \underbrace{\mu L \rho_x}_{\approx 10^{-4}}$$

\Rightarrow after one division:

$$n_{\text{SMMR}} \rightarrow n_{\text{SMMR}} \times 2$$

$$n_{\text{WT}} \rightarrow n_{\text{WT}} \times 2(1 - \mu L \rho_x)$$

\Rightarrow after t divisions:

$$n_{\text{SMMR}} \rightarrow n_{\text{SMMR}} \times 2^t$$

$$n_{\text{WT}} \rightarrow n_{\text{WT}} \times [2(1 - \mu L \rho_x)]^t$$

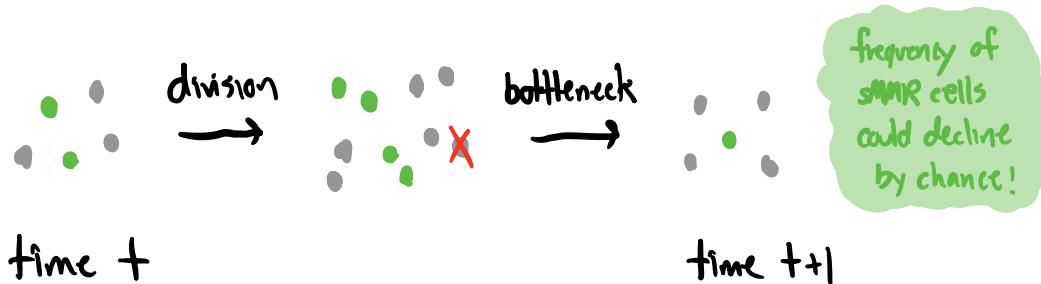
$$\Rightarrow \frac{n_{\text{SMMR}}(t)}{n_{\text{WT}}} \propto (1 - \mu L \rho_x)^{-t} \propto e^{\mu L \rho_x t}$$

i.e., the **super MMR** strain has a small but steady advantage

\Rightarrow natural selection to lower mutation rates

One missing ingredient: population can't double forever...

⇒ e.g. finite resources / predation limits pop'n to size N.

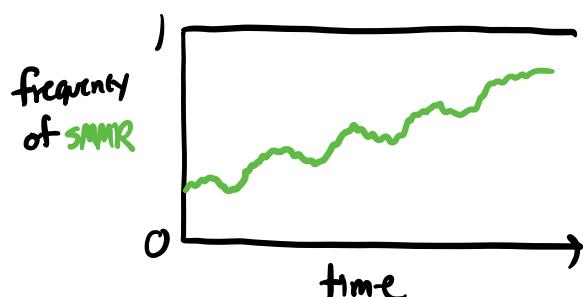


⇒ Simplest model (~"coin flipping")

$$n_{\text{SMMR}}(t+1) \sim \text{Binomial}\left(N, \frac{n_{\text{SMMR}}(t)}{n_{\text{SMMR}} + n_{\text{WT}}(1 - \mu L \rho_x)}\right)$$

⇒ or in terms of frequency ($f \equiv n_{\text{SMMR}}/N$)

$$f(t+1) \equiv \frac{1}{N} \cdot \text{Binomial}\left(N, \frac{f(t)}{f + (1-f)(1 - \mu L \rho_x)}\right)$$



frequency of SMMR
strain undergoes
"random walk" /
"Brownian motion"

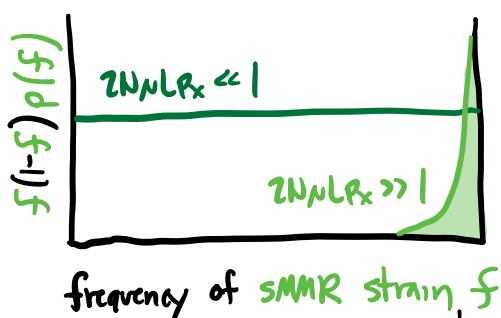
$$\Rightarrow \langle \Delta f \rangle \approx \mu L P_x f(1-f) \Leftrightarrow "F_f" \quad \text{e.g.-lectures 4+5...}$$

$$\text{Var}(\Delta f) \approx \frac{1}{N} f(1-f) \Leftrightarrow "D"$$

Diffusion Equation: $\frac{\partial p(f,t)}{\partial t} = -\frac{\partial}{\partial f} \left[\underbrace{\mu L P_x f(1-f) p(f,t)}_{\text{natural selection } ("F_f")} \right] + \frac{\partial^2}{\partial f^2} \left[\underbrace{\frac{f(1-f)}{2N} p(f,t)}_{\text{genetic drift } ("D")} \right]$

New ingredient: diffusion const depends on f !

$$\Rightarrow @ \text{"equilibrium": } f(1-f)p(f) \propto e^{2N \cdot \mu L P_x \cdot f}$$



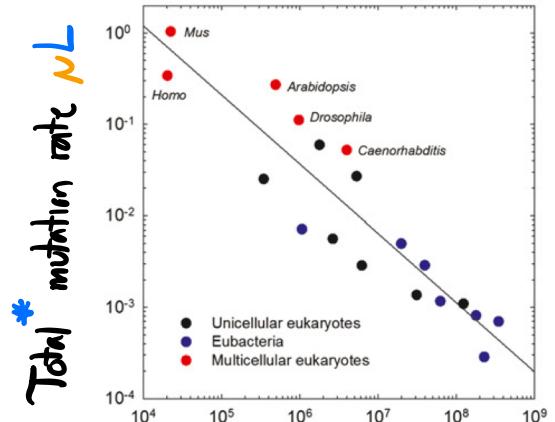
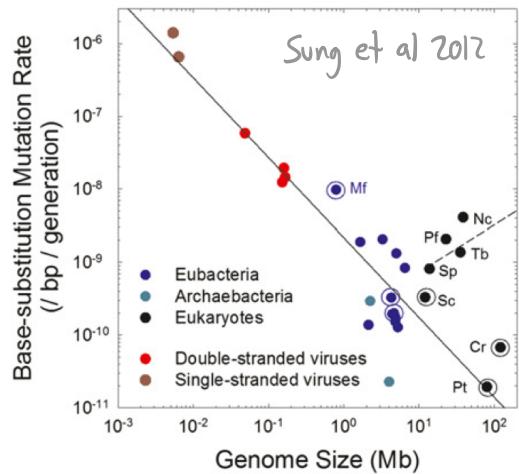
$$-\mu L P_x f \leftrightarrow \text{"energy"} \\ 2N \leftrightarrow 1/kT$$

analogue of
Boltzmann distribution

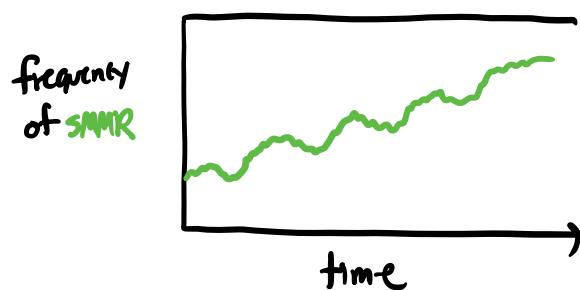
\Rightarrow natural selection is only effective if $N\mu L P_x \gg 1$

\Rightarrow fundamental limit to optimization
of mut'n rates $\mu L \gtrsim 1/N$ ("drift barrier")

\Rightarrow Do real organisms approach this limit?



Drift barrier = one hypothesis for explaining scaling of mutation rates across species...



Intimately connected
to physics of diffusion!

\Rightarrow for additional considerations, see Good & Desai (2016)