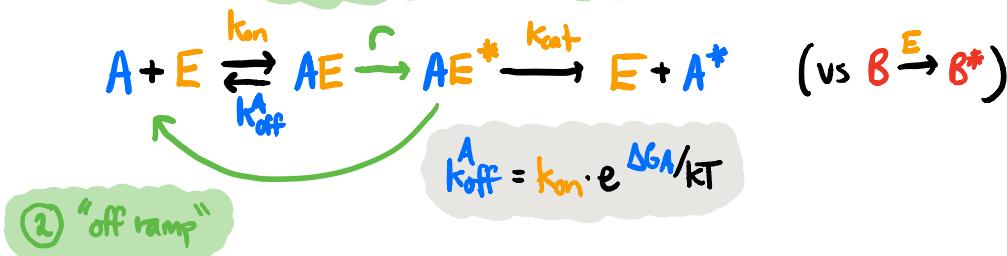


Last time: molecular errors & kinetic proofreading

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!

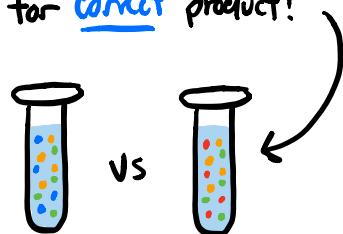
Tradeoffs: ① "wastes" energy

② slower / lower throughput

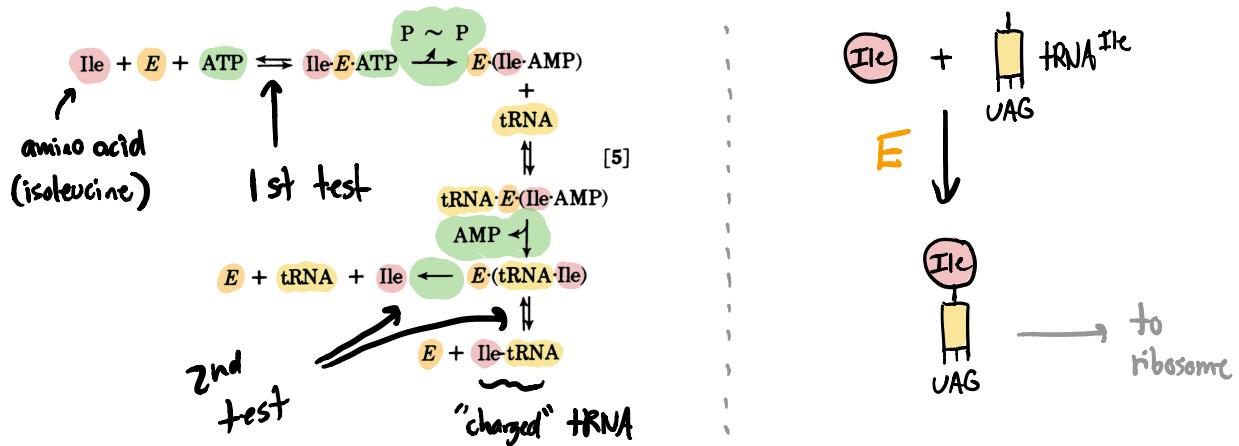
\Rightarrow Hopfield et al '76: test for kinetic proofreading

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

→ should be larger
for errors than
for correct product!



Hogfield 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 $\frac{270}{1.5} = 180$

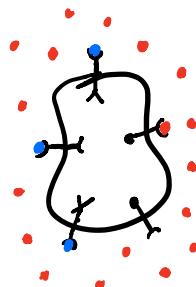
⇒ initial error rate ($Ile + E + ATP \rightleftharpoons Ile-E-ATP$) $\sim 1/100$

⇒ total error rate $= \frac{1}{100} \cdot \frac{1}{180} \sim 10^{-4}$

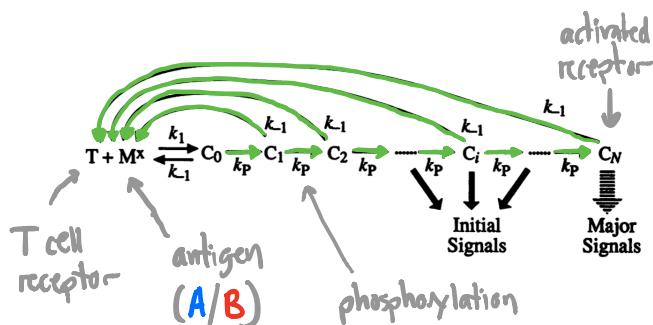
⇒ general idea can be extended to many other contexts

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?

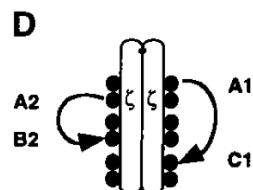


$$\frac{C_N^B}{C_N^A} = \frac{[B]}{[A]} \left(\frac{k_p + k_l^B}{k_p + k_l^A} \right)^{N+1}$$

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

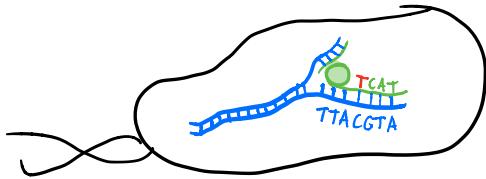
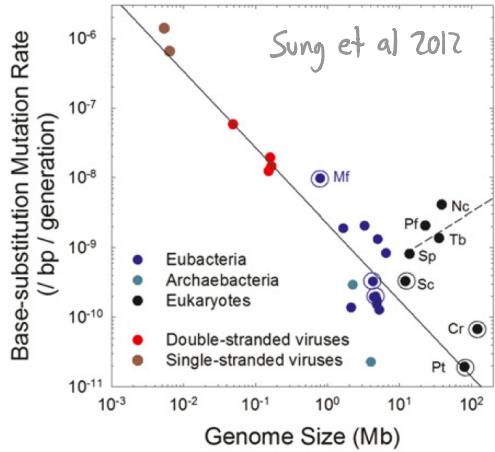
Ellen Neumeister Kersh, Andrey S. Shaw, Paul M. Allen*

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...



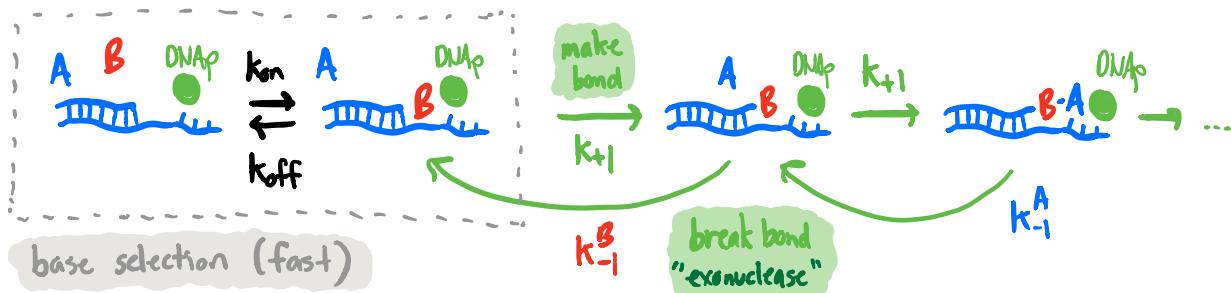
\Rightarrow what do real cells do?

\Rightarrow a little more complicated than above ...

\Rightarrow some key factors:

① DNAP can modify AAG relative to value in sol'n (e.g. exclude H₂O)
(see e.g. Petruska et al '86)

② Many DNAsps also use "exonuclease proofreading"



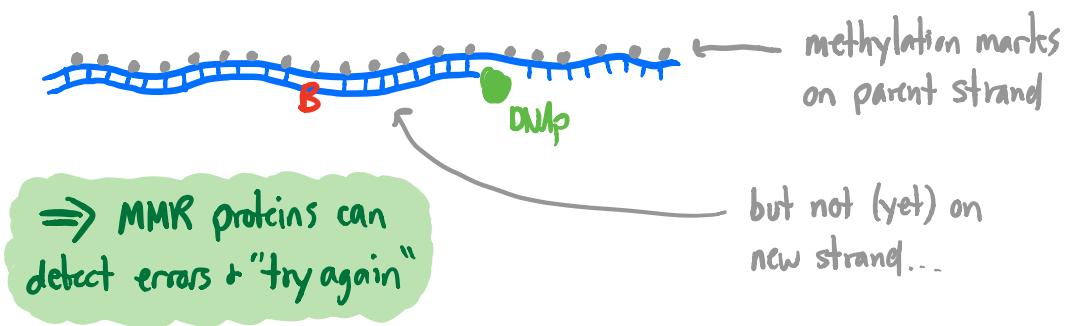
⇒ not technically kinetic proofreading, but similar idea:

(i) Irreversible step + "off ramp" can enhance specificity

(ii) Tradeoffs = "wasted" energy + reduced processivity

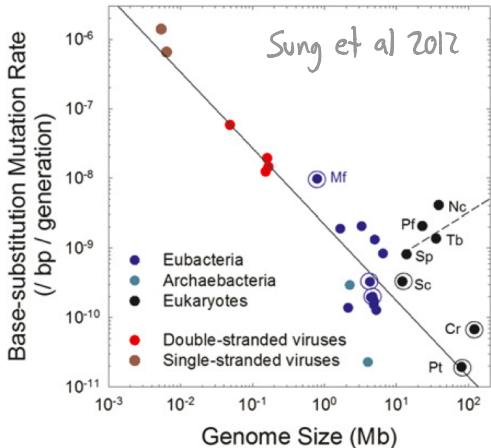
③ Additional error correction mechanisms beyond DNAP

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?

⇒ one possibility: fewer lethal mutations

e.g. E. coli genome:

↳ "essential" sites
(mutation = death)

⇒ 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" ($\sim 1-10\%$ in E.coli)

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

⇒ 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 ($\mu \rightarrow 0$)



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



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

\Rightarrow after one division:

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

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

\Rightarrow after t divisions:

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

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

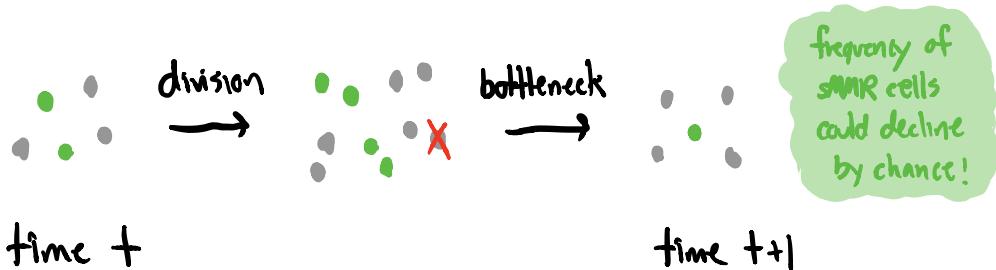
$$\Rightarrow \frac{n_{SMMR}(t)}{n_{WT}} \propto (1 - \mu L p_x)^t \propto e^{\mu L p_x t}$$

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.

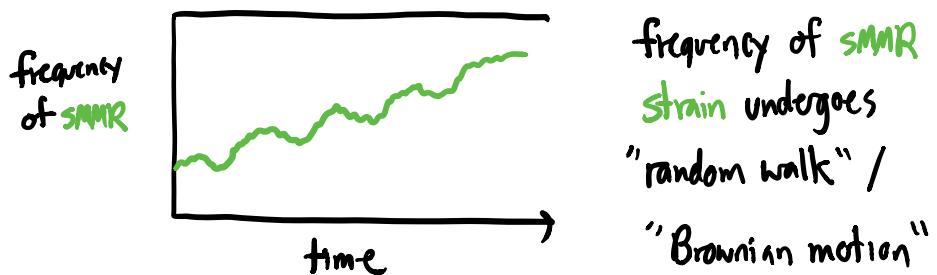


⇒ Simplest model (~"coin flipping")

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

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

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



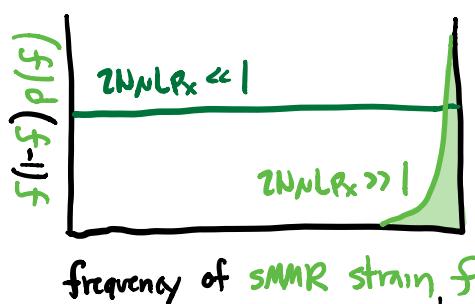
$$\Rightarrow \langle \Delta f \rangle \approx \mu L p_x f(1-f) \Leftrightarrow "F_f" \quad \text{e.g.-lectures 4+5...}$$

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

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

New ingredient: diffusion const depends on f !

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



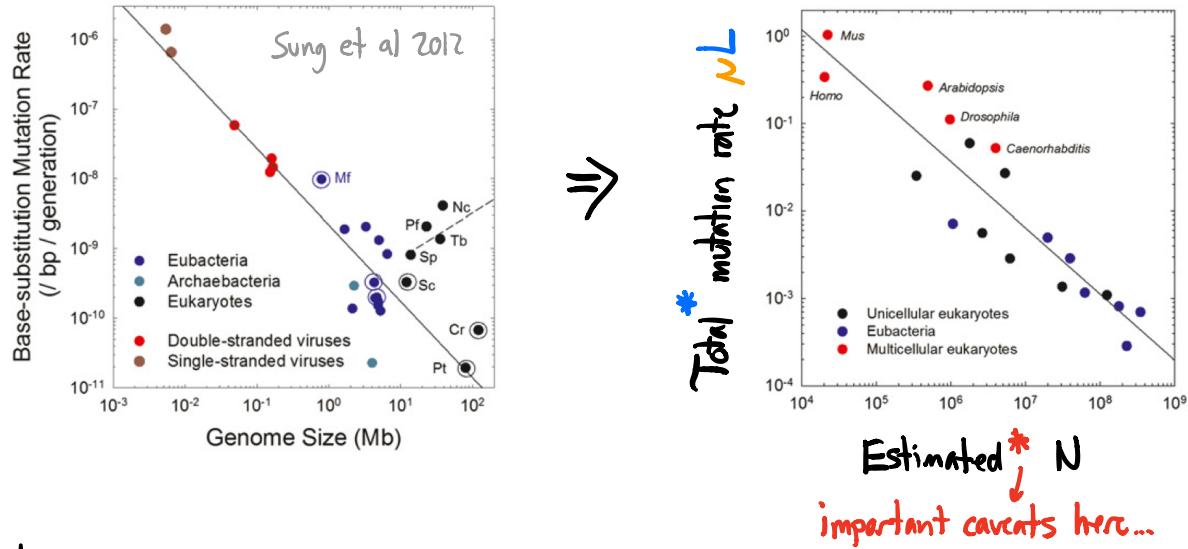
$$-\mu L p_x f \leftrightarrow \text{"energy"} \\ 2N \leftrightarrow 1/kT$$

analogue of
Boltzmann distribution

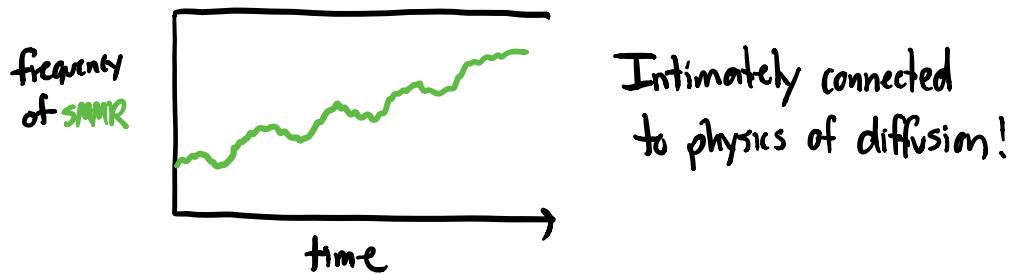
\Rightarrow natural selection is only effective if $N\mu L p_x \gg 1$

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

⇒ Do real organisms approach this limit?



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



⇒ for additional considerations, see Good & Desai 2016