

Last time: How do bacteria follow concentration gradients?



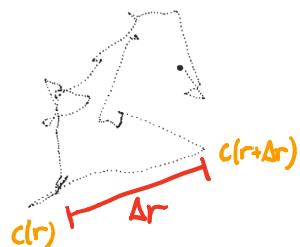
① Works @ low concentrations!

$$[\text{e.g. } C \approx 3 \text{nM} \approx 2 \text{ nm}^{-3}]$$

⇒ discrete molecules matter!

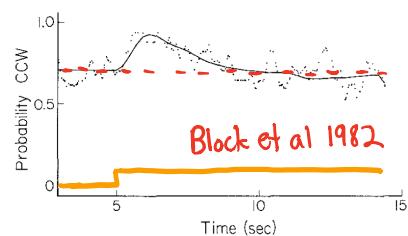
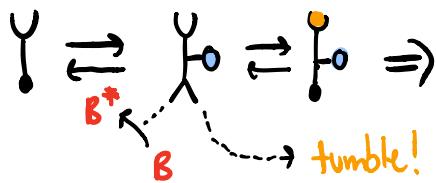
② Burg-Purcell Limit on precision of chemoreception:

$$\frac{d \log C}{dr} \gtrsim \frac{1}{\Delta r} \frac{1}{\sqrt{D_{act}}}$$

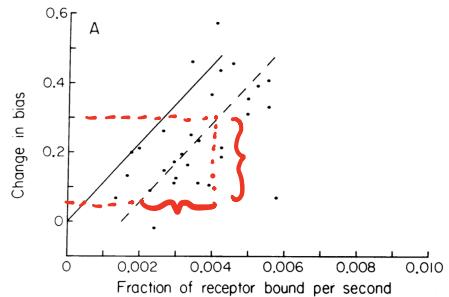


⇒ E. coli must swim to achieve necessary precision!

③ Simple pathways for measuring temporal gradients

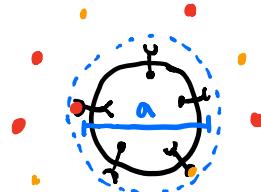


$\Rightarrow$  even single bound receptor  
 $(\sim 1/600)$  can produce  
 large change in bias



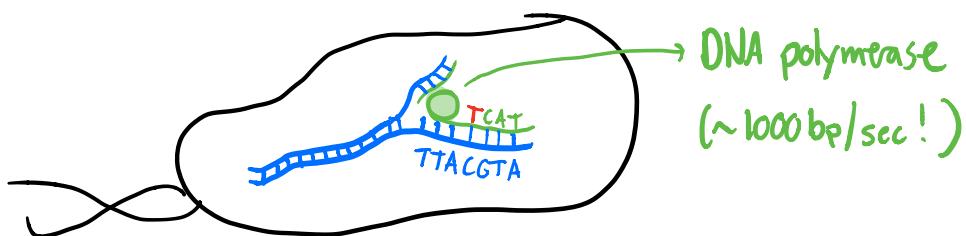
Today: does this **high sensitivity** make  
 cells susceptible to **errors**?

E.g. bind wrong  
 substrate?



$\Rightarrow$  Lots of related **error problems** in cell biology...

## ① Copying genome (DNA) during cell division



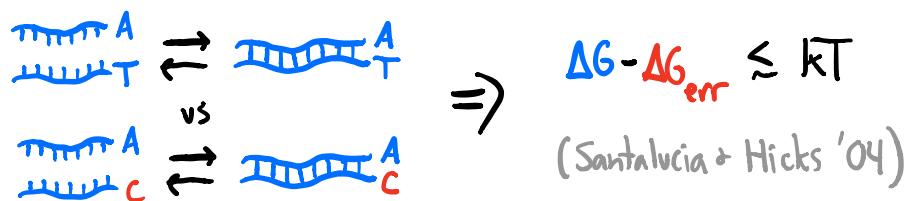
key insight: chemistry (i.e. free energy) favors  
 correct pairs (A-T, C-G)

$\Rightarrow$  errors in n=1 molecule cause permanent change in offspring!

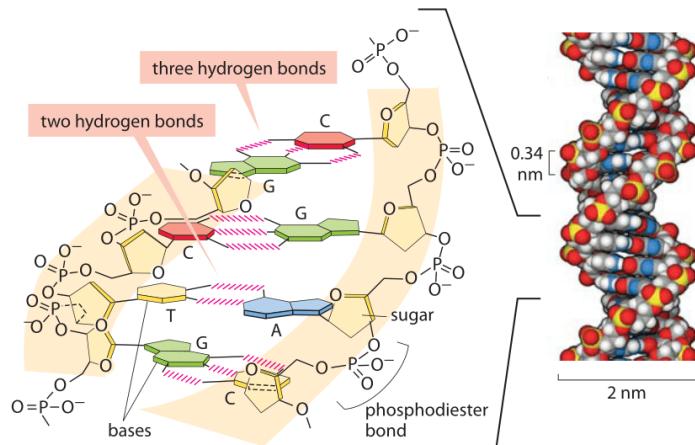
$\Rightarrow$  How strong are these free energy differences?

$\Rightarrow$  still a bit controversial... (Oertell et al 2016)

a) In vitro hybridization experiments:



b) Counting hydrogen bonds



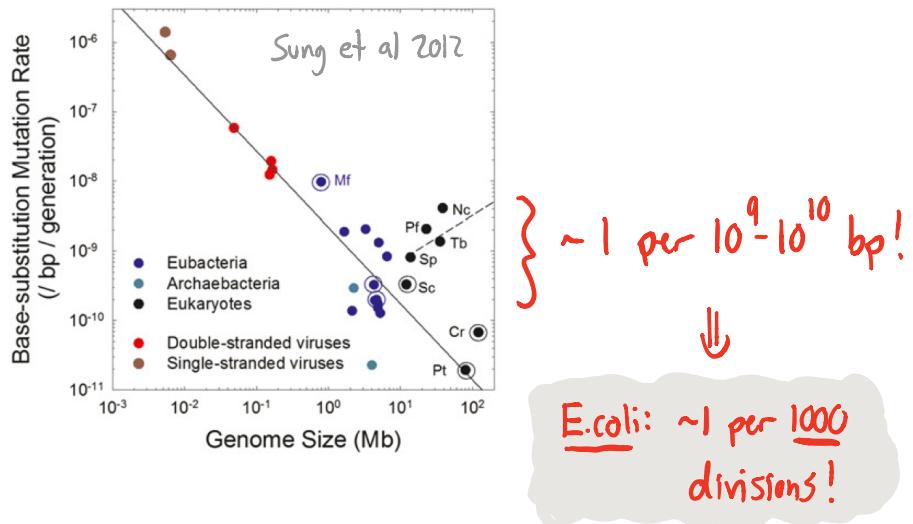
$\Rightarrow$  mispairing  $\approx$  1-2 hydrogen bonds ( $5-10kT$ )

$\Rightarrow$  Naive Boltzmann factor @ equilibrium:

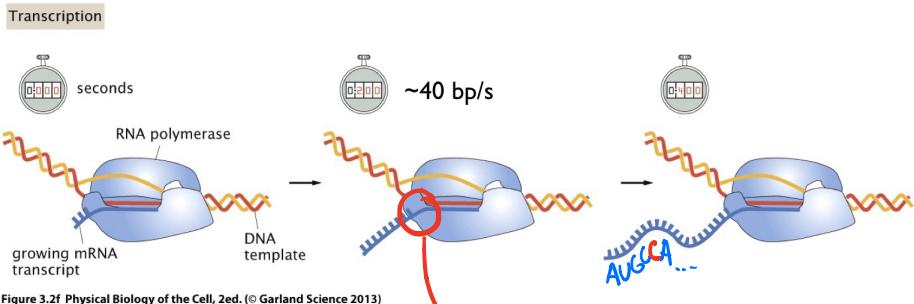
$$\frac{p(\text{error})}{p(\text{correct})} \sim e^{-\frac{\Delta G - \Delta G_{\text{err}}}{kT}} \sim 10^{-1} - 10^{-4}$$

$\Rightarrow > 1$  error every  $10^4$  bp!

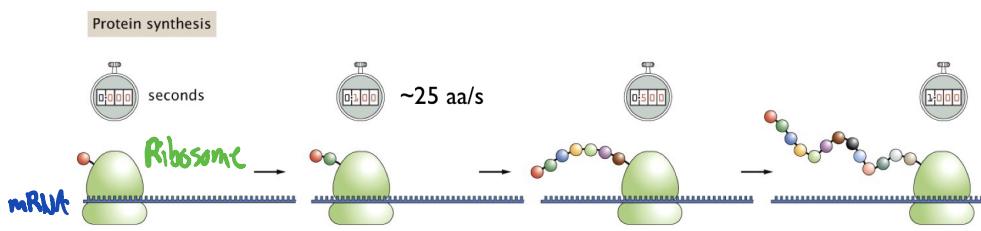
$\Rightarrow$  Actual mutation rates =



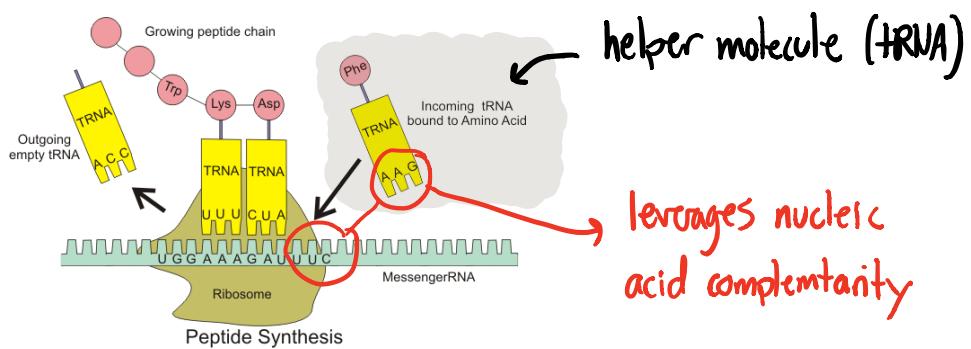
## ② Errors in transcription & translation:



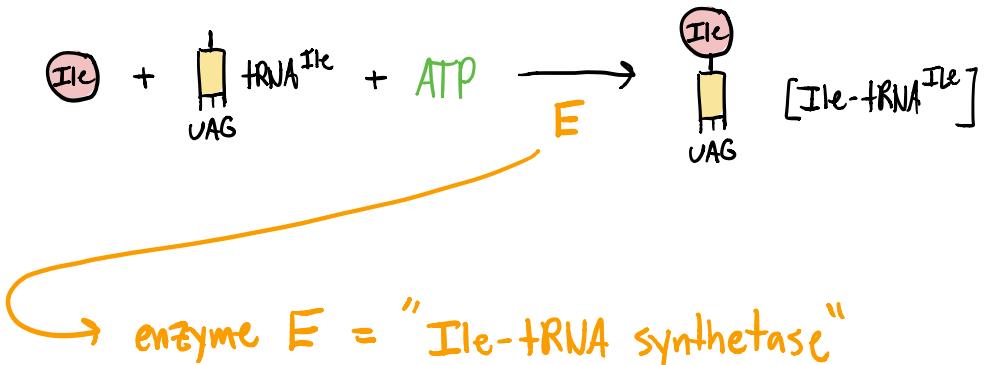
→ leverages similar chemical complementarity as DNA



⇒ How does this work?



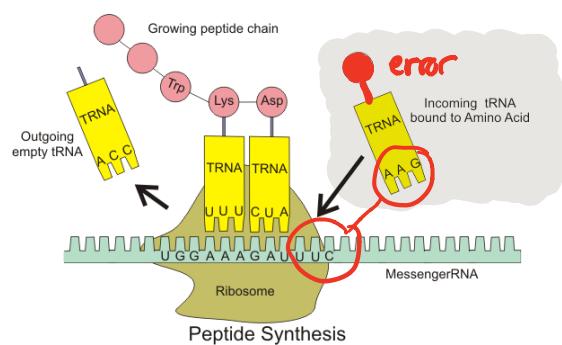
$\Rightarrow$  tRNAs must also be "charged" w/ correct AA!



$\Rightarrow$  tRNA synthetase can also mess up :

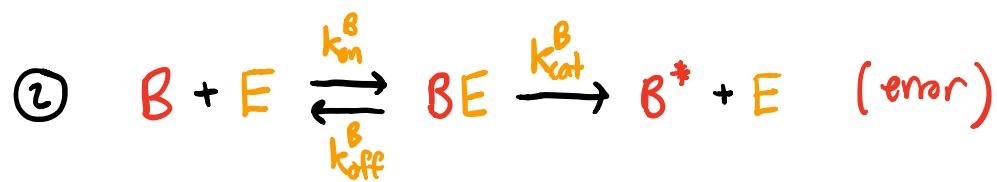
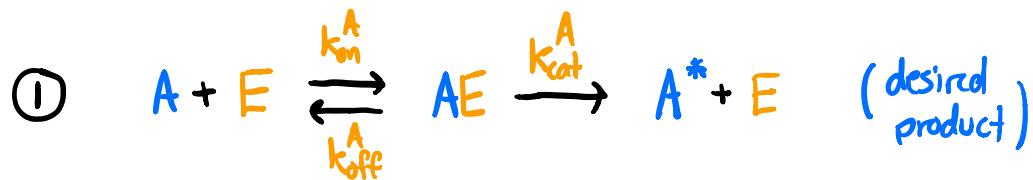


$\Rightarrow$  leads to errors  
@ translation:



Question: what controls these basal error rates  
( + how might cells improve on them? )

Simplest scenario : Michaelis-Menten Reaction  
w/ 2 competing substrates



Rate

Eqns:

$$\frac{d[AE]}{dt} = k_{\text{on}}^A [A][E] - k_{\text{off}}^A [AE] - k_{\text{cat}}^A [AE]$$

$$\frac{d[B E]}{dt} = k_{\text{on}}^B [B][E] - k_{\text{off}}^B [BE] - k_{\text{cat}}^B [BE]$$

$\Rightarrow$  @ steady state :

$$[AE] = \frac{k_{\text{on}}^A [A][E]}{k_{\text{off}}^A + k_{\text{cat}}^A}$$

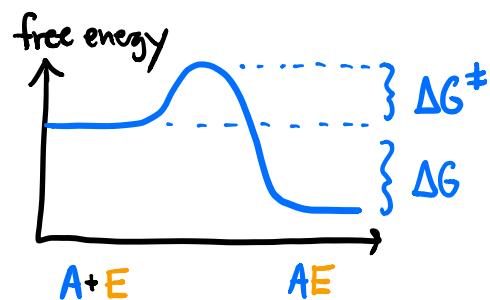
$$[BE] = \frac{k_{\text{on}}^B [B][E]}{k_{\text{off}}^B + k_{\text{cat}}^B}$$

$$\Rightarrow \rho \equiv \frac{\text{rate of error product}}{\text{rate of correct product}} = \frac{k_{\text{cat}}^B [BE]}{k_{\text{cat}}^A [AE]}$$

$$= \frac{[B]}{[A]} \times \left( \frac{\frac{k_{\text{on}}^B k_{\text{cat}}^B}{k_{\text{off}}^B + k_{\text{cat}}^B}}{\frac{k_{\text{on}}^A k_{\text{cat}}^A}{k_{\text{off}}^A + k_{\text{cat}}^A}} \right)$$

$\Rightarrow$  Question: what sets  $k_i^A$  vs  $k_i^B$ ?

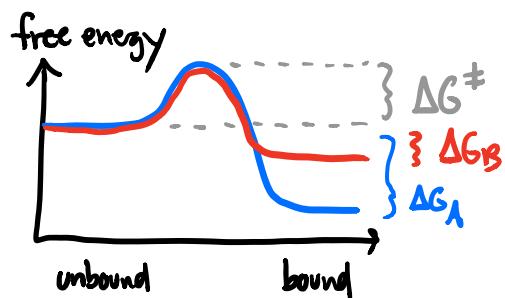
Lecture 6:  
Arrhenius Law



$$\Rightarrow k_{\text{on}}^A \propto e^{-\frac{\Delta G^‡}{kT}}$$

$$k_{\text{off}}^A \propto e^{-\frac{\Delta G^‡}{kT} - \frac{\Delta G_A}{kT}}$$

for similar substrates,  
might expect that:



$$\Rightarrow k_{on}^B \approx k_{on}^A \equiv k_{on}$$

$$k_{off}^B \approx k_{on} e^{+\frac{\Delta G_A}{kT}}$$

$$k_{off}^B \approx k_{on} e^{+\frac{\Delta G_B}{kT}}$$

$$\Rightarrow \text{Similarly for catalytic step: } k_{cat}^A \approx k_{cat}^B \approx k_{cat}$$

$\Rightarrow$  e.g. bonds formed @ different part of molecule than determines enzyme binding.

$\Rightarrow$  e.g. limited by other processes (ATP hydrolysis)

$\Rightarrow$  Putting everything together:

$$\frac{\text{relative error rate, } \rho}{\text{rate}} = \frac{[BE]}{[AE]} = \frac{[B]}{[A]} \times \frac{k_{on} e^{+\frac{\Delta G_A}{kT}} + k_{cat}}{k_{on} e^{+\frac{\Delta G_B}{kT}} + k_{cat}} \geq \frac{[B]}{[A]} e^{-\frac{\Delta G_B + \Delta G_A}{kT}}$$

(lower bound achieved when  $k_{cat} \ll k_{off}^A, k_{off}^B$ )

$\Rightarrow$  big problem if " $\Delta G$ "  $\lesssim 10kT$  & need ultra low error rates

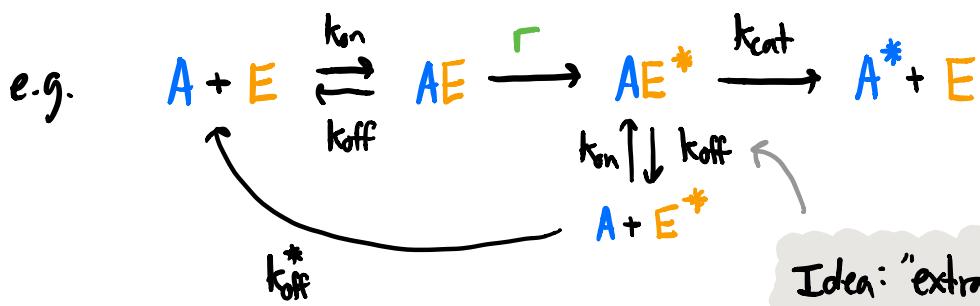
Question: can cells evade this thermodynamic limit  
w/ simple molecular toolkit?

One potential mechanism: "kinetic proofreading"

⇒ originally proposed by Hopfield ('74) + Ninio ('75)

⇒ later extended to many different settings

Basic idea: add a "spurious" irreversible step  
after binding but before catalysis:



Idea: "extra time"  
to test  $k_{off}$ ?

$\Rightarrow$  now relative error rate,  $\rho \equiv [BE^*] / [AE^*]$

New rate equations:

$$\frac{d[AE]}{dt} = k_{on}^A[A][E] - k_{off}^A[AE] - r(AE)$$

$$\frac{d[AE^*]}{dt} = r(AE) + k_{on}^A[A][E^*] - k_{off}^A[AE^*]$$

$$\frac{d[E^*]}{dt} = -k_{on}^A[A][E^*] + k_{off}^A[AE^*] - k_{off}^*[E^*]$$

+ versions w/ B substrate ...

$\Rightarrow$  solve @ steady state (algebra exercise)

$\Rightarrow$  when  $r, k_{cat} \ll k_{off}$ :

$$[AE] \approx \frac{k_{on}^A}{k_{off}^A} [A][E]$$

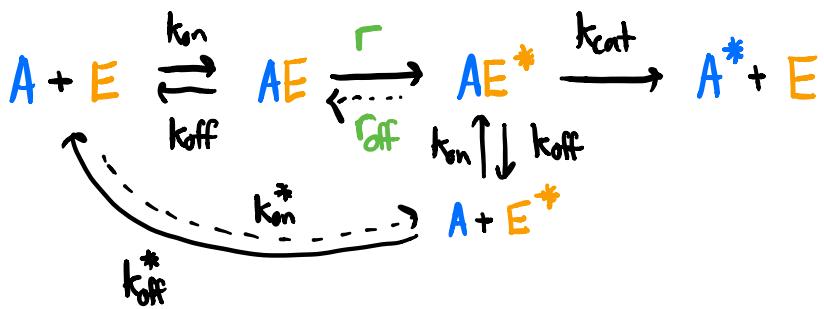
$$[AE^*] \approx \frac{k_{on}^A}{k_{off}^A} \left( \frac{r}{k_{off}^A} \right) \left( 1 + \frac{k_{on}^A[A]}{k_{off}^*[E^*]} \right) [A][E]$$

↑      ↑  
extra factor of  $k_{off}^*$ !

$$\Rightarrow \text{relative error rate } \rho = \frac{[BE^*]}{[AE^*]} = \frac{[B] \left( 1 + \frac{k_{on}[B]}{k_{off}^*} \right)}{[A] \left( 1 + \frac{k_{on}[B]}{k_{off}^*} \right)} e^{-\frac{2(\Delta G_A - \Delta G_B)}{kT}}$$

$\Rightarrow$  i.e. squares error rate ( $10^2 \rightarrow 10^4$ ,  $10^4 \rightarrow 10^{-8}!$ )

$\Rightarrow$  Irreversibility was crucial! E.g. reversible version:



$\Rightarrow$  Same calculation yields  $\frac{[BE^+]}{[AE^+]} = \frac{[B]}{[A]} e^{\frac{\Delta G_A - \Delta G_B}{kT}}$

$\Rightarrow$  how to ensure irreversibility ( $r_{off} \ll r, k_{cat}$ )?

$\Rightarrow$  burn free energy! e.g. ATP hydrolysis:



$\Rightarrow \Delta G_{ATP}$  not used to build product,  
but to limit reversibility!

$\Rightarrow$  i.e. "wasted" energy is feature not bug!

### Kinetic Proofreading



Benefits: enhances specificity above thermodynamic limit

Costs: ① slower processivity:

$$\Rightarrow \text{slowdown} = \frac{[AE^+]}{[AE]} = \frac{r}{k_{off}} \approx 1$$

② "wastes" energy!  $A+E \xrightarrow{k_f} AE \xrightleftharpoons{c} AE^+ \xrightarrow{k_{off}} A^+ + E$

$$\frac{\text{energy use}}{\text{per product}} = \frac{r[AE]}{k_{cat}[AE^+]} = \frac{k_{off}}{k_{cat}} \gtrsim 1$$

$\Rightarrow$  Hopfield: can be used as test for proofreading!

## Regular M.M.



vs



$\Rightarrow$  same used energy  
for correct vs error

## Kinetic Proofreading

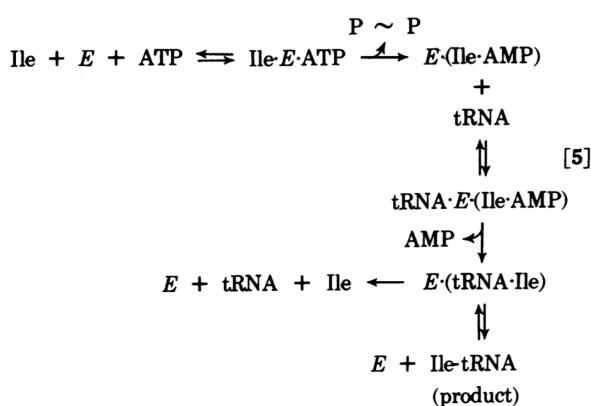


vs



$\Rightarrow$  more burned energy  
per error product!

$\Rightarrow$  applied to tRNA charging:



Found: ~1.5 ATP

per correct charging

vs

~270 ATP for error!

$\Rightarrow$  consistent w/ proofreading hypothesis...

$\Rightarrow$  potential explanation for complicated rxn.