Lecture 10: Kinetic proofreading

Chap 9 of Alon

9.1 Introduction

- Recognition of molecule X by its interaction partners
- Problem of molecular recognition of a target despite the background of similar molecules: How can a biochemical recognition system pick out a specific molecule in a sea of other molecules that bind it with only slightly weaker *affinity*?
- Diverse molecular recognition systems in the cell seem to employ the same principle to achieve high precision: **kinetic poofreading** (Hopfield 1974)
- Two cases of kinetic proofreading considered:
  - Reading the genetic code during **translation**
  - Molecular recognition in the **immune system**

Kinetic proofreading idea: Example Picasso

- Recognition of Picasso lovers in an art museum by 'kinetic proofreading'
- Inflow of visitors: 50% of visitors are Picasso lovers, 50% are non-lovers
  - A lover wants to stay in the Picasso room 10 minutes
  - A non-lover wants to stay in the Picasso room 1 minute
- Steady state: Picasso room has 10 times more lovers than non-lovers
- Enhanced enrichment (kinetic proofreading): close the two-way entrance door, open a one-way exit door
  => after some time, the only ones remaining in the Picasso room are Picasso lovers; so the enrichment for lovers is much higher than 10-fold

9.2 Kinetic proofreading of the genetic code can reduce error rates of molecular recognition

- **Translation**: a ribosome produces a protein by linking amino acids one by one into a chain (Fig 9.1)
- **Genetic code**: the mapping between the 64 codons (triplets of nucleotides of mRNA) and the 20 amino acids (Fig 9.2)
- A wrong tRNA can attach to the codon; such translation errors occur at frequency of about 10^{-4} which is much better quality than what one would expect from the affinity differences between different tRNAs (each codon has its own specific tRNA); the improved accuracy is due to kinetic proofreading

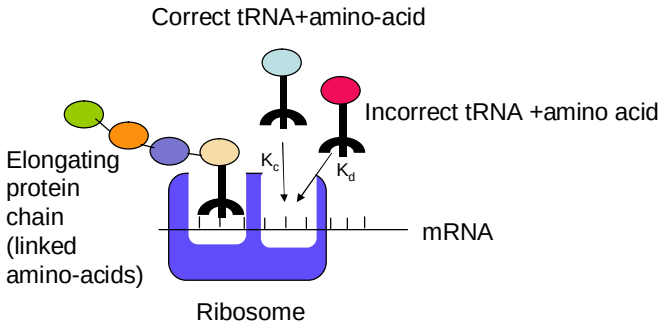


Fig 9.1: Translation of a protein at the ribosome. The mRNA is read by tRNAs that specifically recognize triplets of letters on the mRNA called codons. When a tRNA binds the codon, the amino acid that it carries links to the elongating protein chain. The tRNA is ejected and the next codon is read. Each tRNA competes for binding with the other tRNA types in the cell. The correct tRNA binds with dissociation constant K_c, whereas the closest incorrect tRNA binds with $K_d > K_c$.

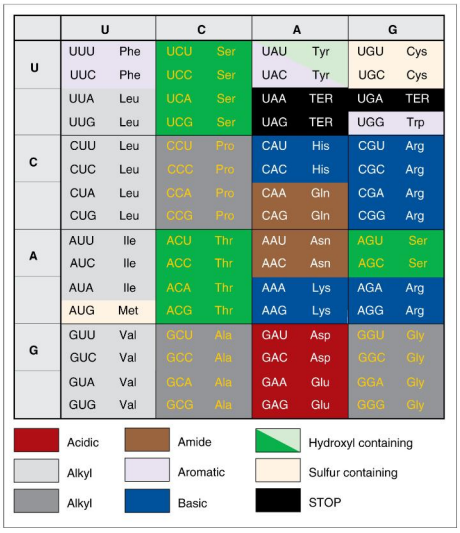


Fig 9.2: The 'universal' genetic code. Shading indicates polar requirement (PR)1: lighter shades (black text), PR , 6 (hydrophobic); medium shades (yellow text), PR 5 6–8 (medium); darker shades (white text) PR . 8 (hydrophilic). From Knight Freeland and Landweber, TIBS 24, 1999.

9.2.1 Equilibrium binding cannot explain the precision of translation

- The simplest model of recognition process: equilibrium binding
- Notation
  - C = the next codon on the mRNA (also: the concentration of C)
  - c = the tRNA for codon C (also: the concentration of c)
  - k_c = on-rate of codon C binding c
  - [cC] = the resulting cC complex when C binds c
  - k_c' = off-rate of unbinding c from C
  - v = probability per unit time that the amino acid attached to RNA c will be covalently linked to the translated protein chain

Equilibrium binding (cont.)

- Equilibrium process

$$c + C \underset{k(c)'}{\overset{k(c)}{\leftrightarrow}} [cC] \overset{v}{\rightarrow} \text{correct amino acid}$$

- At steady state (one may neclect v as $v << k_c, k_c'$):

$$cCk_c = [cC]k_c'$$

$$\Rightarrow [cC] = cC/K_c \text{ where dissociation constant } K_c = k_c'/k_c$$

- Incorporation rate of the correct amino acid

$$R_{correct} = v[cC] = vcC/K_c$$

- In addition to the correct tRNA c, the cells contain different tRNAs that carry the other amino acids and compete for binding to codon C

Equilibrium binding (cont.)

- Notation
  d = incorrect tRNA whose affinity to codon C is the highest
  k_d, k_d' = on-rate and off-rate of C binding d
- Steady state

$$d + C \underset{k(d)'}{\overset{k(d)}{\leftrightarrow}} [dC] \xrightarrow{v} \text{incorrect amino acid}$$

=> $[dC] = dC/K_d$ where dissociation constant $K_d = k_d'/k_d$

=> $R_{wrong} = vdC/K_d$

- $K_d > K_c$ (as d is the incorrect tRNA) => $R_{wrong} < R_{correct}$
- Resulting **error rate**

$$F_0 = R_{wrong}/R_{correct} = vdCK_c/vcCK_d \approx K_c/K_d \quad (\text{as } d \approx c)$$

Equilibrium binding (cont.)

- The on-rates for both c and d are limited by diffusion and are about the same: $k_c = k_d$ (Appendix A)

=> $F_0 = R_{wrong}/R_{correct} = K_c/K_d \approx k_c'/k_d'$

that is, it is the off-rate k_d' that distinguishes the correct codon from the incorrect one: $k_d' >> k_c'$

- Experimental: $K_c/K_d \sim 10^{-2}$ (c.f. observed error rate is 10^{-4} – a large discrepancy!)

9.2.2 Kinetic proofreading can dramatically reduce the error rate

- tRNA c undergoes a chemical modification: c binds to C and then c is converted to c*, with reaction rate m; **this reaction is virtually irreversible**
- The modified tRNA c* can either donate its amino acid to the protein chain or fall off of the codon C (reaction rate l_c') *without being able to mount back any more*

=> $c + C \underset{k(c)'}{\overset{k(c)}{\leftrightarrow}} [cC] \overset{m}{\rightarrow} [c^*C] \overset{v}{\rightarrow}$ correct amino acid

$\downarrow l_c'$

$c + C$

Reducing the error rate (cont.)

- The concentration of [c*C] is given by the balance of the two processes with the rates m and l_c' (neclect v that is small): $m[cC] = l_c'[c^*C]$ which yields steady-state solution

$$[c^*C] = m[cC]/l_c'$$

$$R_{correct} = v[c^*C] = vmcC/(l_c'K_c)$$

- The same applies for d (conversion to d* etc)
- The off-ratio of c* and d*:

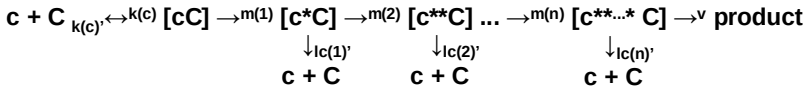
$$l_d'/l_c' = k_d'/k_c' \approx K_d/K_c$$

=> $R_{wrong} = v[d^*C] = vmdC/(l_d'K_d)$

=> $F = R_{wrong}/R_{correct} = (K_c/K_d)(l_c'/l_d') = (K_c/K_d)^2 = F_0^2$

Reducing the error rate (cont.)

- Thus the proofreading step can reduce from the equilibrium recognition error rate $F_0 = 1/100$ to much better error rate $F_0^2 = 1/10000$
- Even higher level of fidelity can be attained by linking together n irreversible (or nearly irreversible) proofreading processes:



$F = F_0^{n+1}$

9.3 Recognizing self and non-self by the immune system

- Fig 9.3
- Fig 9.4
- Kinetic proofreading increases fidelity of T-cell recognition: analysis of how the error rate is improved is given in Chapter 9.3; technicallly quite similar to analysis of transcription in Chap 6.2

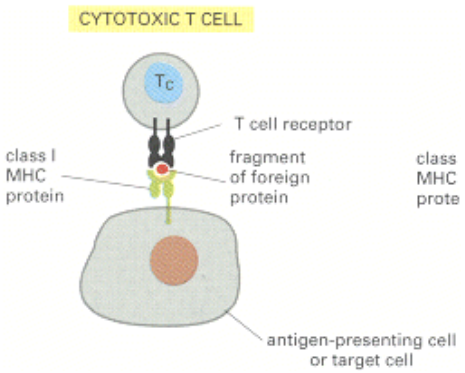


Fig 9.3: Recognition of foreign peptides by T-cells. Target cells present fragments of their proteins bound to MHC proteins on the cell surface. Each T-cell can recognize specific foreign peptides by means of its T-cell receptor. Recognition can result in killing of the target cell by the T-cell.

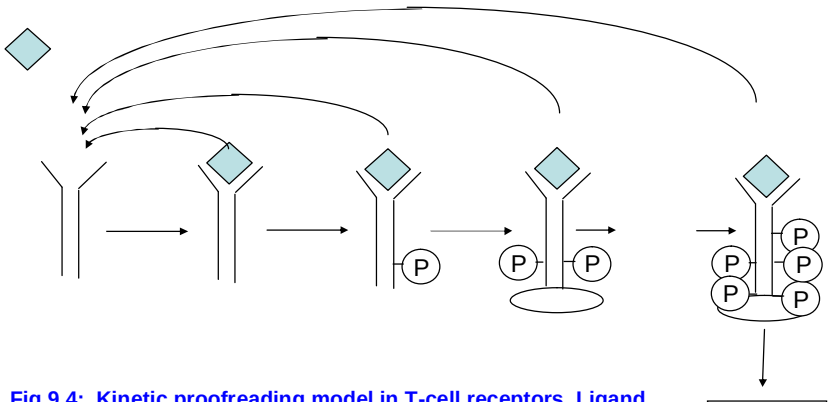


Fig 9.4: Kinetic proofreading model in T-cell receptors. Ligand binding initiates modifications to the receptors. When sufficient modifications have occurred, signaling pathways are triggered in the cell. At any stage, ligand can dissociate from the receptor, resulting in immediate loss of all of the modifications. The series of modifications creates a delay between ligand binding and signaling. Only ligands that remain bound throughout this delay can trigger signaling.