411-3 2009 Lecture notes

I. First general topic in the course will be mutation (in broadest sense, any change to an organism's genetic material). Intimately intertwined with this is the process of DNA replication, which I want to review in first part of lecture. Know most of you have covered mechanism of replication in or other classes ...

PP 1
Sources of mutation:
1. DNA replication errors
2. DNA damage
3. Genetic events (e.g., transposon insertion)

PP

Outline of lecture

PP

Basic attributes of genetic processes (replication, transcription, translation)

1. speed- 500 b/sec for replication
2. fidelity- 10^{-10} error frequency for E. coli replication
3. regulation- e.g., replication initiation tied to growth rate- won't talk about-covered in text

PP
Themes of today's lecture:

II. Chromosome replication- as most of you know two replication forks (= growing regions)
PP : Chromosomal replication <u>bidirectional</u>- draw- focus on middle part- progress of one fork
PP: Draw fork proceeding- review what's needed to go from left to right (point)
-steps

PPs-
go through one by one

helicase
SSB
DNA polymerase III

Write on OH: Properties of DNA polymerases

1. **5'–>3' only**
2. **can't initiate de novo**

RNA priming and Okazaki fragments
Mop-up functions: DNA polymerase I and DNA ligase

Complex- Fig 1.12 (3^{rd})- large complex of proteins

Complex mechanism with many parts- more in text!- needed to ensure speed and regulation.

Last function of replication complex different- ensures highly accurate base polymerization- Proofreading

a. **PP**-Need: have tautomers ~10^{-5} of the time, so how get 10-10 error rate?

b. 3' (or 3'->5') exonuclease – can be attached to DNA polymerase or a separate protein which is part of replication complex

PP showing mech
Evidence- biochemical and genetic
genetic- property of mutant lacking proofreading function? mutator

Extended: Mutator- 10^{-10} ->10^{-7} (can't fully eliminate)
If too high- DEATH (error catastrophe- approx 1 lethal mutation/replication round)

If overactive?-Antimutator (10^{-10}->10^{-11})
What are they?

These are **slow growing**? (think mistakes when not)-like someone always double-checking if turned off the stove when trying to leave the house
antimutator = slow-growing; **trade-off between speed and accuracy**

Illustration- predictions for lab-rushing to fill out predictions at last minute- know more likely to make errors than in done ahead of time with time to check

PP:

	error frequency
base selection-	10–5
editing	10–4
mismatch repair	10–1
	10–10

If DNA 1 meter in diameter, machinery about the size of a FEDEX truck. Replication of *E. coli* genome a 250 mile trip, speed of 375 mph, with a delivery every 4 inches. One mistake (delivering the wrong package) every 500 miles.

Come back to the fundamental trade-off between speed and fidelity

1. Anti-mutator polymerase as extreme in spectrum- makes fewer mistakes but grows slow, not optimal. Cells are so slow and careful that they never get anywhere

2. Other end of extreme are RNA bacteriophages
   -fast and efficient but make enormous number of errors
   PP-smallest phages-stripped down-
   PP life cycle- a single infected cell gives off 10,000 phage in ~30 min- 100X typical phage
   -what's amazing incredibly high mutation rate- (between 70-90% are mutant in an essential function and can't give a productive infection!)
   Mutation frequency 10^{-3} to 10^{-4} per base replicated

Key: RNA replicase doesn't have a proofreading exonuclease function (and mismatch repair doesn't work on RNA). So example in which accuracy has been sacrificed for the sake of speed. This life cycle strategy somwhat analogous to that used by most insects- make lots of progeny and even if some or most die, still enough make it to perpetuate species

ACCURACY THROUGH REDUNDANCY

Proofreading during replication –> high fidelity is attained by building a redundancy into the process- correct pairing checked twice, once in original nucleotide selection, then again by proofreading exonuclease. This is the general logic used in biological systems to increase fidelity of a process- multiple independent mechanisms to check that mistakes ain't been made. Briefly mention two other illustrations of this use of redundant checking to decrease error rate- both from protein synthesis (translation).

Translational Fidelity

PP- need for high fidelity- although not as high as replication
   1000 amino acid protein- even if 10^{-3} error rate, still get mistakes in 60% of proteins; 10-4 observed in cells

PP- elongation steps of protein synthesis- pp 92; Fig 2.27 (S and C, 3rd).

Key recognition steps-both have proofreading mechanisms:

1. attachment of amino acid to tRNA
2. binding of aa-tRNA at appropriate codon

OH- Two step mechanism to charge aatRNA single enzyme (aa tRNA synthetase)

OH- Two amino acid recognition steps in Ile-tRNA biosynthesis
each step gives ~100X discrimination- allows subtle distinctions (e.g., between val and ile)
-alternative would be that every aa-AMP gets tRNA

2. More subtle - Kinetic proofreading during elongation step
<u>key thing is exit pathway</u>

OH- have to maintain codon-anticodon interaction during time required for GTP cleavage

DEMO Peace sign vs Fist (pops up show) (analogous to good vs bad codon recognition)

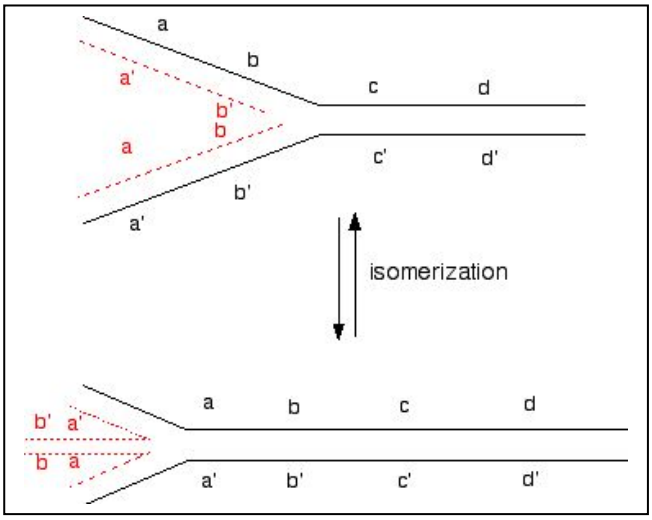
Problem is that fist transiently mimics peace sign-(like tautomerization) - show

Kinetic proofreading- introduces pause between initial binding and response
-doesn't affect recognition of peace sign; -helps prevent recognition of fist

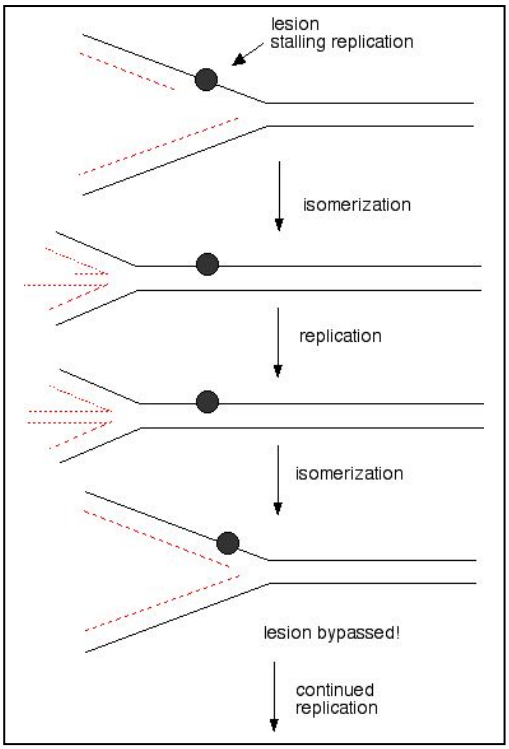
-COST-Takes energy to introduce delay- GTP cleavage in case of translational proofreading

Hyperaccurate TL mutants grow slowly

Three examples of how high fidelity in a genetic process is achieved through redundancy- one in replication, two in protein synthesis. In each of these examples, original, provisional choice is checked in a second step- if wrong, aborted.



Isomerization of replication fork, say if stalls when encounters damage it can't replicate over- focus on top strand same as one of new strands



Lesion stalling replication- show bypass without repair of damage