Myron Goodman: Fidelity

So... why is base mutation important?

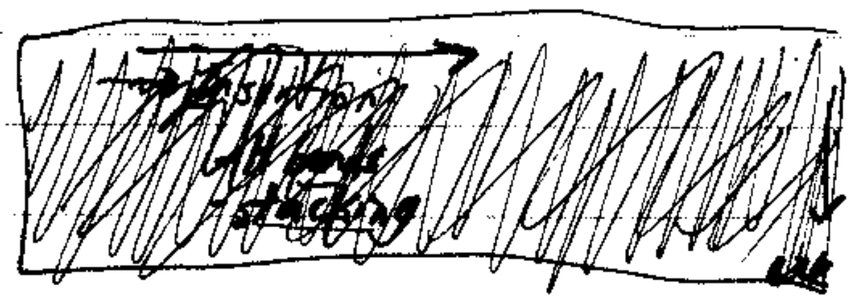
Assays for fidelity?

Gel kinetic assay

- sequence context
- generalize assay for proofreading

Presteady state fluorescence

Reactions contributing to fidelity



① Insertion

- fidelity is independent of absolute nucleotides and dependent on relative concentrations.

- H bonds
- base stacking (may govern sequence context affects)

② Extension vs. excision

- proofreading depends on concentration of NEXT nucleotide (because this affects timing of next insertion)

- suggests excision recognizes ss vs dsDNA in region near site

Experiments

Gel Kinetics

velocity at site 3 = $V_{2\to3}$ = $\frac{I_3}{I_2} K_{off\ at\ 2}$ (I Band in Gel) w/o exonuclease

want $\frac{V_{max}}{K_m}$

Pol α
- ☺ some transversions easily made (A-A e.g.)
- ☹ some very rarely made (C-C e.g.)

Rev Trase (w/ DNA template)
- ☺ not so error prone for first insertion
- ☹ but prob. of extending mismatch is quite high

Competition Between Extension & Excision

Pol III Holoenzyme
- α
- ε
- θ
- 5

2 Amino Purine (=P)
- fluorescent ... more so on own than in DNA
- polarization decay highest on own

- atmospheric removal

4.7	P-T in GC rich	∴ strandedness of region important
11	P-C in GC rich	
37	P-T in AT rich	
47	P-C in AT rich	