GORDON RESEARCH CONFERENCE ON MUTAGENESIS

Plymouth State College June 26-July 1, 1994

John M. Essigmann (Chair) and Philip C. Hanawalt (Vice-Chair)

Monday Morning: DNA Polymerases: Their Actions on Damaged and Undamaged Templates

Bernard Strauss (Chair)

1. Myron Goodman: Biochemical basis for DNA synthesis fidelity
2. Kathleen Dixon: UV mutagenesis in mammalian extracts
3. Graham Walker: SOS mutagenesis

Thomas Kunkel (Discussion Leader)

Monday Evening: How Proteins View and Process DNA Damage

John Gerlt (Chair)

1. Gregory Verdine: Recognition and repair of aberrantly methylated DNA
2. Daniel Treiber: Recognition of metal-DNA complexes by HMG-box proteins

Bruce Demple (Discussion Leader)

Tuesday Morning: Mismatch Repair

Josef Jiricny, (Chair)

1. Peter Karran: Mismatch recognition, DNA damage tolerance and cancer susceptibility
2. Robert Lahue: Mismatch repair proteins in yeast
3. Richard Fishel: Genome stability and *hMSH2*

Richard Kolodner (Discussion Leader)

Tuesday Evening: DNA Repair in Eukaryotic Systems

Jan Hoeijmakers (Chair)

1. Louise Prakash: DNA repair in yeast
2. Mutsuo Sekiguchi: Mammalian enzymes that prevent occurrence of spontaneous mutations

Jacques Laval (Discussion Leader)

Wednesday Morning: Endogenous Mutagenesis

Leona Samson (Chair)

1. Jeffrey Miller: Spontaneous mutations and rearrangements
2. Lawrence Loeb: Mutagenesis by oxygen free radicals
3. Mark Meuth: Mutator genes in human colorectal carcinoma

Susan Wallace (Discussion Leader)

Wednesday Evening: Transcription Coupled Repair and Mutagenesis

Philip Hanawalt (Chair)

1. Christopher Selby: Mechanistic studies of the MFD protein
2. Isabel Mellon: Transcription-dependent repair in E. coli
3. Brian Donahue: Transcription of damaged DNA in vitro

A. Steven Leadon (Discussion Leader)

Thursday Morning: Lesion Structure and Mutagenesis

Robert Fuchs (Chair)

1. Christopher Lawrence: Mutagenic properties of UV photoproducts in vivo
2. Edward Loechler: What controls the mutagenic specificity of carcinogens?
3. Lawrence Marnett: Mutagenesis by malondialdehyde: From adduct structure to mutation

Arthur Grollman (Discussion Leader)

Thursday Evening: Mutational Origins of Genetic Disease

Thea Tlsty (Chair)

1. Cheryl Walker: Genetic determinants of susceptibility to chemical carcinogenesis
2. Carl Anderson: DNA-damage and the DNA-activated protein kinase

Raymond Monnat (Discussion Leader)

Friday Morning: Adaptive Mutagenesis

Patricia Foster (Chair)

1. Susan Jinks-Robertson: Adaptive mutagenesis in yeast
2. John Roth: Tests of a new explanation of selection-directed mutagenesis

Bryn Bridges (Discussion Leader)

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

WHITAKER COLLEGE OF HEALTH SCIENCES AND TECHNOLOGY
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June 1, 1994

Jonathan Eisen
Stanford University
Dept. of Biological Sciences
Herrin Hall #356
Stanford, CA 94305

Dear Jonathan,

I am very pleased to inform you that you have been admitted to the 1994 Gordon Research Conference on Mutagenesis. Phil Hanawalt and I have worked hard on the program and are looking forward to a great meeting. The Mutagenesis conference is typically oversubscribed, so if your plans change, I would greatly appreciate it if you could let me know that you will not be joining us by FAX at 617-258-8676.

I look forward to seeing you in June.

Sincerely,

John M. Essigmann

B. Strauss

SUGGESTS THAT PEOPLE have ignored structures of DNA polymerases and damaged DNA.

Damaged Templates

① What is the relationship between lesion bypass and frameshift

Bypass mechanisms

a) recombination
b) branch migration
c) misalignment & slippage at the lesion
d) translesion synthesis

Frameshift Assays

Lact w/ frameshifts in pyrimidine tracts

② diff. polymerase diff. freq. of mutation w/ UV damage

③ deer. to deer. % mutants

④ EXO⁻ diff. pattern

Myron Goodman - Reactions in Replication

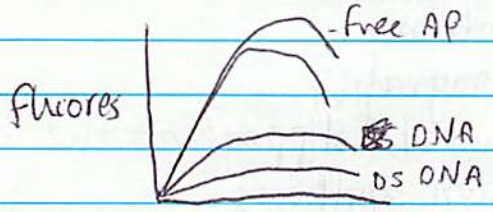
insertion → extension

↓

excision

Method

- fluorescence of 2-AP in DNA



2AP = T

2AP = A wobble

2AP = C mispair

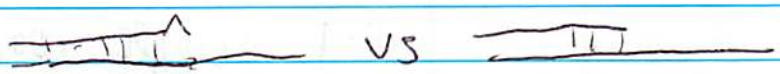
- use decay of fluorescence to look at ns time scale
- anisotropy decays
  - if bound to DNA doesn't lose isotropy fast

Questions

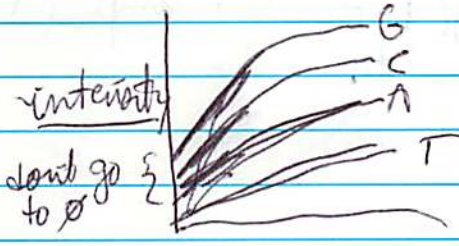
① affect of sequence content on exonuclease

- GC content v. important (stab. mispairs)

② structure
- "melted" vs "annealed"
- suggests exonuclease removed melted DNA



REMOVAL OF 2-AP

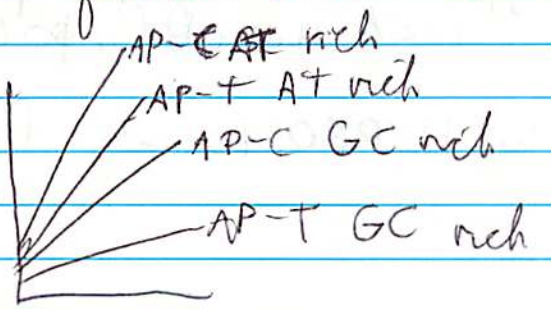


- this correlated w/ base pair energy (Tm)

T	31.4	Tm
A	28	
C	24	
G	20	

G,C,A ... does not goes to zero; fit double exponential
T ... goes to zero; single exponential

removal of AP-T in AT rich area is ~~slower~~ faster than AP-C in GC rich



Uses fluorescence to distinguish among equilibrium states of polym. vs exonuclease

Shapes of curve

AP-T ... single exponential

$$Y = \text{ampletude}_1\left(1-e^{-K_1 t}\right) + \text{amp}_2\left(1-e^{-K_2 t}\right)$$

A_1 K_1 A_2

+	0
A	0.081
C	0.17
G	0.35

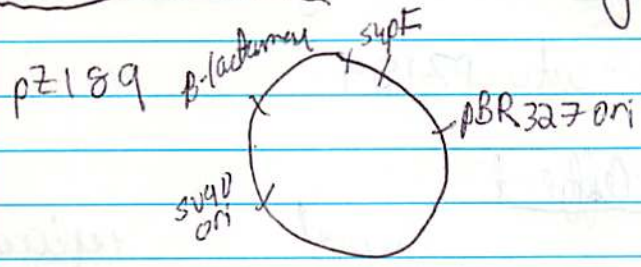
↑ stability of BP?

D. wood BAR / H. Echols

- bypass of lesion only in presence of recA, umuDC, ϕ (III)

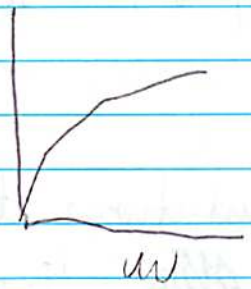
- w/ HSP60 to seq-fold umuC

Kathleen Dixon UV mutagenesis



mammalian cells (HeLa) → extracts → add
- pZ189
- T antigen *
- dNTPs
- NTPs

→ transform → XD sup mutants → sequence



① UV damage

② photolyase reduced freq. of mutation suggesting CPD cause the mutations seen

③ mutation spectra
- most at TC/CT

④ bypass occurs
... what about evidence for blockage

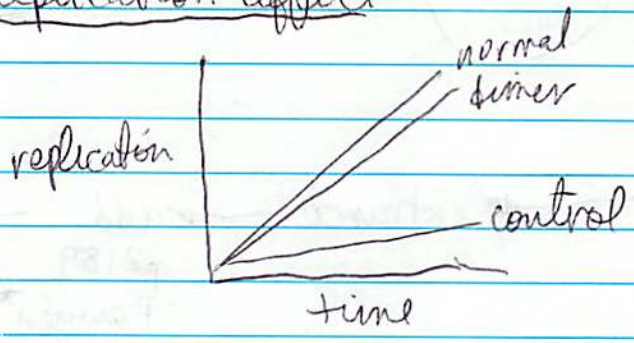
- What about selection bias?
- What about evidence for replic. blockage

Specific lesions - $\hat{TT}$ - single dimer

① $\hat{TT}$

oligo inserted ... into PZ189

Replication Effect



replication products still contain dimer

Mutations

- >3000 colonies ... no mutation
- fits w/ v. few mutations at TT site

②

BPDE

- most mutations at GC positions
- many $\frac{G}{C} \rightarrow \frac{T}{A}$

- 20 mutants - all at pos 123 - all G→T

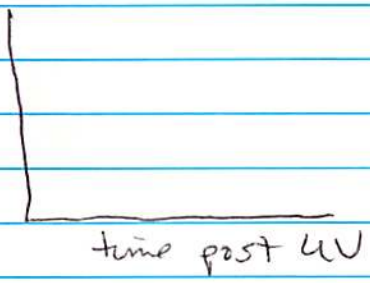
Induction

- cells $\xrightarrow{+/-\ UV}$ ~~AAAAA~~ add UV vector → delet mutants

how long incubate

- some incr. in mut. freq. w/ UV irrad of host cells

Replication Timing



- suggests hSSB plus phosphorylation is V. important for replication

Conclusions

Scheal

- Deamination of C in dimers - dependent on time
- ung dependent

cI
p22

Graham Walker - SOS

Ecoli

- sos+ mutagenesis− = umuD umuC
  umuA umuB

recA
↓ssDNA
recA*
↳ lexA cleaved
↳ umuD made
↳ umu cleaved

- sequence of umuDC showed sim. to lexA; cI
- suggested cleavage in recA pathway
- evidence that intact umuD is an inhibitor of mutagenesis

- umuC similarity ... do sequence stuff
  - revI in yeast
  - impB
  - mucB
  - samB

RecA role

② may bind umuC ...

① targeting

② DC ~~need~~ operate on recA filaments

PolI - not required
PolII - maybe - prob. not
PolIII - ??? - maybe - prob. yes

GROEL = HSP60
- many mutants deficient in UV-mutagenesis
- maybe involved in setting up umu for cleavage

DNAK -
- not required

UmuD
D - 15 KD intact
D' - 12 KD cleaved
- dimer
- D & maybe D' interact w/ recA

12 monocysteine mutants
- correlated cleavage w/ mutability
- purified
- 2ary prediction

T Kunkel

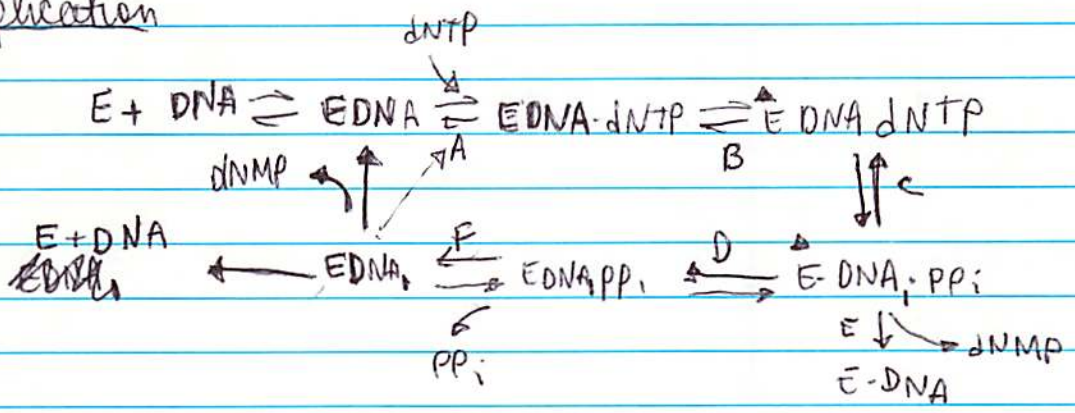
Questions

Polymerases

-Sequence Context-

Replication

see Modrich



Strand Bias

1. sequence context
2. which polymerase
3. selection
4. repair
5. damage

B vs others

B - non-processive for big regions

- doesn't bypass O-6-me-G

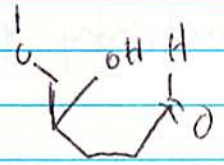
- processive for small-gaps

Lecture Notes

Pol B deletion
- no phenotype even in some doubles

John Gerit

Enzymatic Reaction in DNA Damage Repair



aldehytic abasic site

FPG protein

Greg Verdine

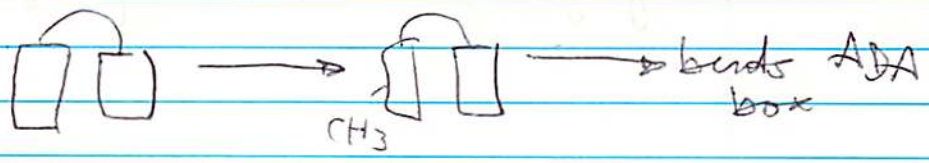
methylation -- variety of agents

ADA - suicide methyl transfer

① C-terminal cysteine S attacks C terminal of O-6-me-G

② N-terminal domain C - attacks CH_3 on S-methyl phosphotriester

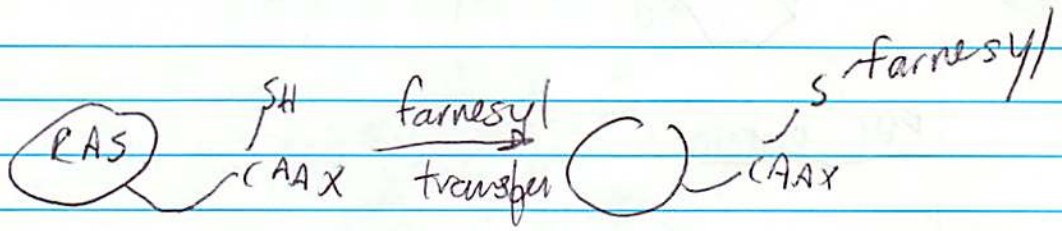
③ gene activation ... "ADA" box



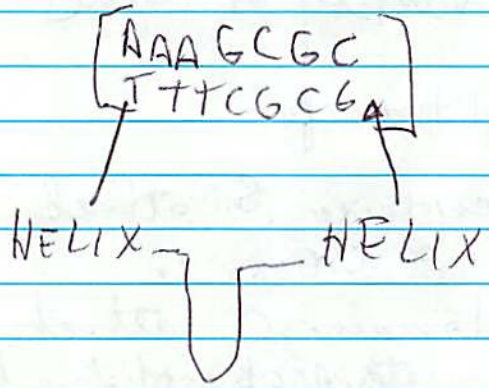
DAN DAN
DAN

N-terminus

- binds Zn (4 cysteines)
- Zn plays a role in activity & structure



ADA Box



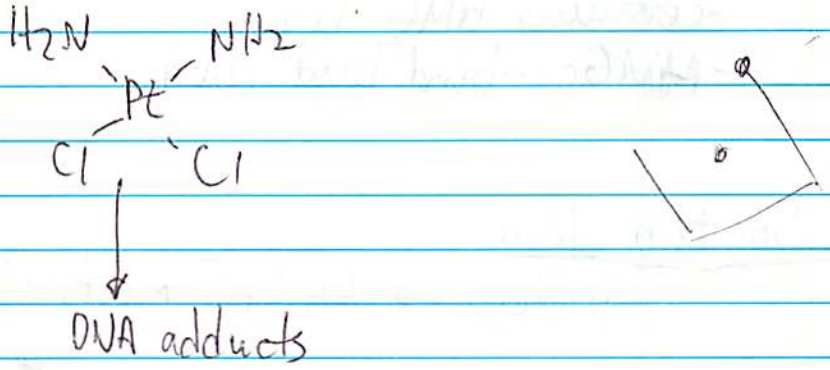
normal HTH protein only H2 interacts w/ DNA

ALKA

- recognizes two diff. methylated bases

Dan the Man

Cisplatin



BAD - detected [illegible]
protein that bound cis-plat adducts

MODELS

(1) Prot is repair protein
- unlikely bec. adducts that bound are not repaired well

(2) prot. blocks repair by blocking adducts

(3) transcription competition

Used cDNA expression to pull out SSRP-1

SSRP-1

- contains HMG box
- HMGs - bind bent DNA
-

Southwestern

proteins → PAGE → nitrocellulose
↓
probe w/ DNA

① two proteins
- HMG-1
- other ?? 97 KDa = hUBF

HUBF

- rRNA tx factor
- binds rRNA promoter
- binds cisplatin v. tightly
- competition of CP-DNA w/ rRNA region occurs

B. Dimple

- photolyase

Sancar ... binds only damaged strand

- ADA -

① crystal of C-term

Broad-recognition

① AP endo

commonality a gap

- can recognize many things

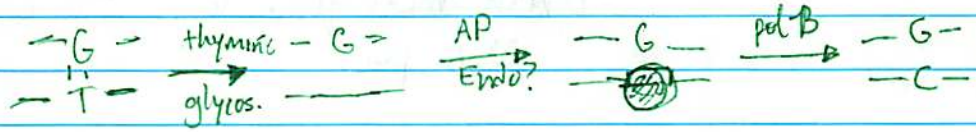
② endo III

③ $f_{pg} = mut M$

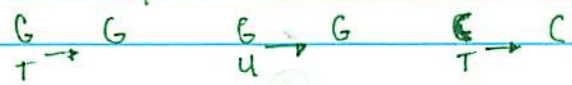
Jiricny

Mismatch Repair
Recognition
① "damage"
② strand

G≠T
- important in methylated genomes
- deamination
- 5-me-C -- hotspots of mutation
- Tom Brown using SV40 w/ mismatches showed that GT was repaired much to GC



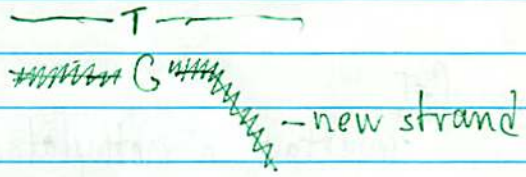
- Purified enzyme ... can cut GT & others



- Recognition
  - suggest T comes out of major groove

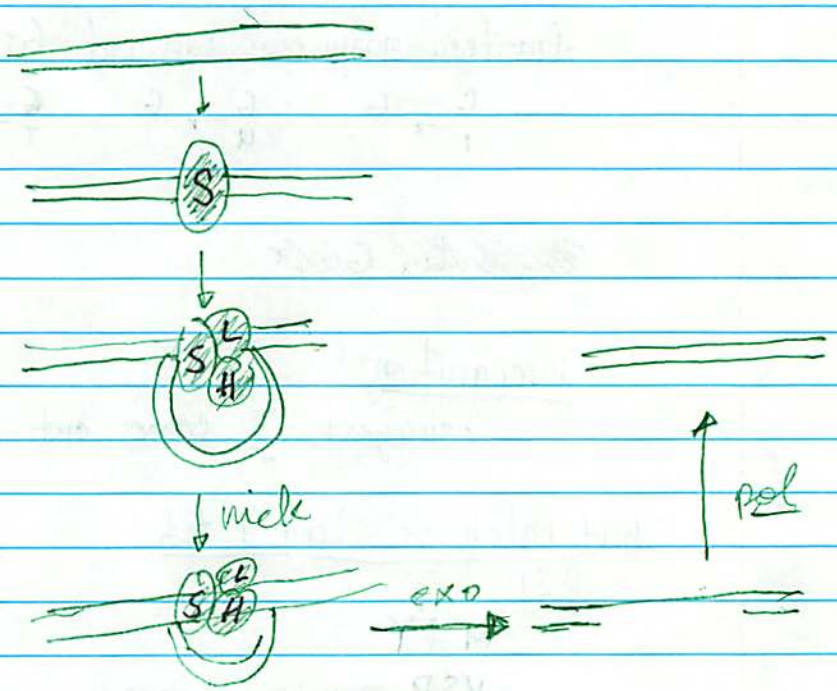
Short Patch vs. Long Patch
① SP
- ~~Mut~~ Y
- VSP = endonuclease

(2) Long patch
- 1-2 kb resynthesized
- replication errors



- suggests 5-me-C is a hotspot because of competition betw. SP & LP

- Dam methylase lags ~2 min behind DNA pol.



PMS1 - 1st Euk

DUG1 & REP3 - 1st ~~a~~ mammalian

Biochemistry

① purified mismatch binding prots
② microsequence
③ one = hMSH2 = GTBP
two = ??? - no homology

Lahue

Yeast Mismatch Binding Activities
① Single base mismatches
② multibase mispairs (inserts/deletes)

Indels Mismatches

① Proks =
- in E. coli < 5nt heterologues
· corrected by MutHLS
- > 5 nt mismatches have no binding to muts

② Eukaryotes
- correct insertion mispairs of all sizes
- may explain stabilization of repeats
- MSH2 recog. small indels
- nothing for bigger

Experiments
① test binding of yeast extracts to ~~bigg~~ mismatch
- MSH2 binds

② test binding to large inserts (5 nt)
- not depend on MSH1-4
- not affected by SS-DNA
- IMP insertion mismatch binding protein

③ stem loop
- Petes has data which suggests DNA stem-loops are pen substrates for gene conversion
- IMP ~~not affected~~ does not bind stem-loops

⑤ dr binding independent of sequence context

In vivo correction of large mismatches

① ADE8 heteroduplexes on plasmid
- in ADE2 mutants ... color ~~selection~~ scoring possible

② 7 bp inserts APPEAR to be corrected well

MSH3 -- mild mutator phen.
--- homeologous recombination
--- not known to bind mismatches

#19

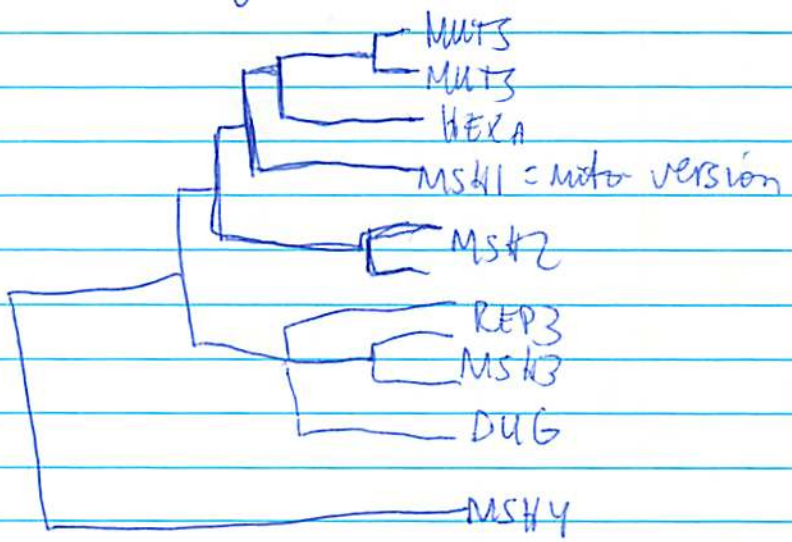
Fishel-

Development of Colon cancer
- multiple steps to metastasis
- too high for spontaneous mutation
  Directed Evolution
  - higher mutation frequency
  - chromosome instability

Recombination & Gene Conversion

- msh-2 .. removed gradient of gene conversion in ARG-4

- cloned human homolog



- involved in colon cancer
- ~~was~~ mapped to same locus

Another loci
- chromo 3
- ~~hPMS1~~
- hMLH1

Peter K. = Methylation

O-6-Me-G

- pairs w/ T
- leads to G→A transition
- repaired by methyltransferase (MGMT)
- MEX⁻ = deficient in MGMT
  - = sensitive to O-6-Me-G
  - = select for MNNG, MNU resistance
    - leads to tolerance
    - resistance do methyl in DNA
    - resistant to O-6-Me G
    - can replicate w/ O-6-Me G (no blockage)

MISMATCH REPAIR
- = dam⁻ mutants are MNNG sensitive
- = MNNG resistance due to mutations in MUTS & MUTL
- suggests mismatch repair is involved in killing cells in dam⁻ cells - possibly by ...??

- dam⁻ recA⁻ cells are inviable

- microsats unstable in GT mismatch repair mutants

EMICS

Local Sequence context of GT

GT binding prot
- binds slipped mispaired intermediates
- mutants have mutator phenotype
- mutants confer resistance to MGMT

Kolodner & discussion

① mismatch repair in recombination does it regulate genetic exchange

② proks vs euks

③ how many MLH, MSH

④ other pathways

Distance From Initiation Site

- ~~affects mismatch repair~~

- likelihood of conversion affected by mismatch repair

- possible because invading strand doesn't go all the way in

Recombination & Mismatch

Does overexpression of MMTS affect recomb.

① freq of recomb betw close markers incr. in mismatch repair mutants

② S. typh & E. coli only recomb. in mismatch repair mutants

③ mutLSH affects recA

MSA3
-ATP not reqd. for binding

Jan Hoeijmakers - Repair

NER in mammals

PIBIDS

Photosens — Impaired Intelligence - dysmyelination
Ichthyosis — ↓ Fertility
Brittle hair — ↓ Growth

NO ↑ CANCER

Excision Repair

Two types -
- tx coupled
- global

ERCC6 --
- have called the yeast gene RAD23
- CS-B
- yeast mutant deficient in tx-coupled repair
- no incr. UV sensitivity
- but incr. recovery time

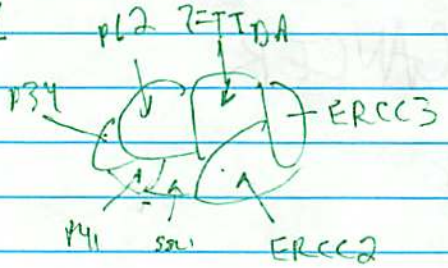
RAD23
- ubiquitin-like fusion protein
- cloned HHRAD23A, HHRAD23B
- binds XPCC protein
- involved in overall repair

- HHR23B -- maps to same region as XPCC
- no correction of any human syndrome

ERCC3

- dominant mutants wipe out tx & repair in microinjections

Complex



5' direction helicase

Propose disease Transcription Disorder

Louise Prakash - Post Replication Repair

"Post replication repair"
① error free
② mutagenic bypass

Yeast
RAD6 : ubiq. conj. enzyme
RAD18 : binds ssDNA

RAD6
RAD18

Error Free → RAD5

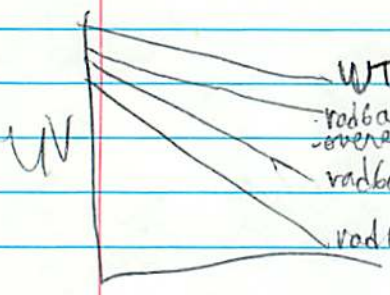
Mutagenic → REV3 : v. similar to DNA pol ζ = deletions not lethal
REV1
REV6
REV7

- UV sens. of rad5 rev3 doubles similar to RAD6; RAD18 sing

but what if other factor overabundant?

Ⓐ RAD6 + RAD18 : coimmunoprecipitate each other
- most/all RAD18 precips w/ RAD6 antibody
- v. tightly associated (as by SDS tx. complex)
- interact directly w/o other factors (using Ab assay) ...

② rad6 Ala-88 ~ · ubiquitin conjugation defective
~ can still interact w/ RAD18
WT ··· overexpression leads to semi-dominant
rad6ala88 overexpression rad18 of UV survival in ~~rad6Δ~~ WT
rad6ala88 background
rad6Δ



- P32 labeled ssM13 DNA -- binds RAD18 not RAD6

- P32 labelled dsM13 DNA ··· doesn't bind either

- ~~RAD~~6 - RAD18 complex is retained on ssDNA agarose column

- suggests RAD6-RAD18 complex β(e) to target ubiquitination to damaged regions

RAD5

- ssDNA depend. ATPase
- no detectible helicase activity
- suggests it is involved in copy choice synthesis

- described T. Petes RAD5 affect on microsatellite stability

connection between mismatch & recomb.

ΔRAD5 --- incr. stability of tract length
--- same spectrum

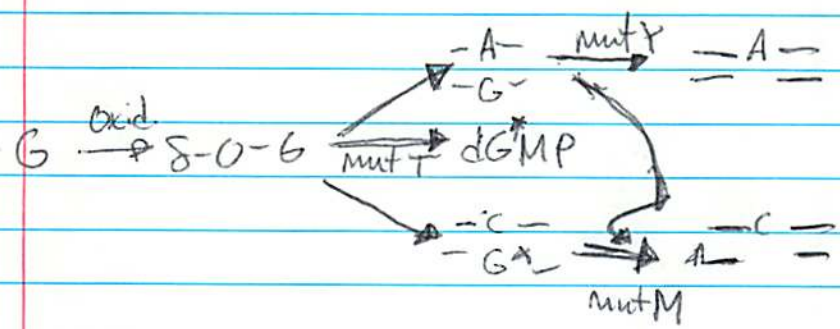
Sekiguchi

Spontaneous Mutagenesis in E. coli

Pre-replication	mut T mut M, mut Y
Replication	dnaE dnaQ
Post-replication	mutS mutL mut H uvr D

Mut T in mammals

- 8-oxodGTPase activity — 8-O-G
- 15 KD



distribution of mutations in rpsL

	WT	mut T
AT→GC	14	0
GC→AT	7	0
GC→TA	2	0
GC→CG	2	0
AT→TA	4	0
AT→CG	2	19

AT→CG incr. in mutT

CG→AT incr. in mutY/M

8-O-GTPase activity in human cells
1. Fractionate
2. purify
3. microsequence
4. cDNA
5. not v. similar to E. coli

Compare w/ other mutT related proteins
KELQEE

Predicted 2ary structure
- conserved region in helix

Express cDNA in ~~MMTT~~ mutT - E. coli

- Gene is 9Kb = chromo 7
- Screen population for MTH mutation
- Mouse KO

Jacques Laval

FPG protein
- Zn finger required for glycosylase & deoxyribose excision but NOT for all of abasic excision activity

AlkA

Deoxyinosine - Me - Products

alkA+ vs alkA- mutation frequencies in lac, rif, ... much the same

not due to error prone evolution

Loeb

Depurination 3.6×10^{-11} /nucl/sec

Multiple Mutations & Cancer
- what are sources?
- change sources NOT repair

Oxidative Damage
Protein
RNA
DNA - 70-100 species


TIM McBride

Mutations by O_2 damage

#1) C → T
2) G → C
3) G → T
4) hot spots -- suggest due to damage hot spots
5) CC → TT

CC → TT
- Nakazawa PNAS 91:360
- Miller JMB 182:45
} - CC → TT in sun-exposed skin

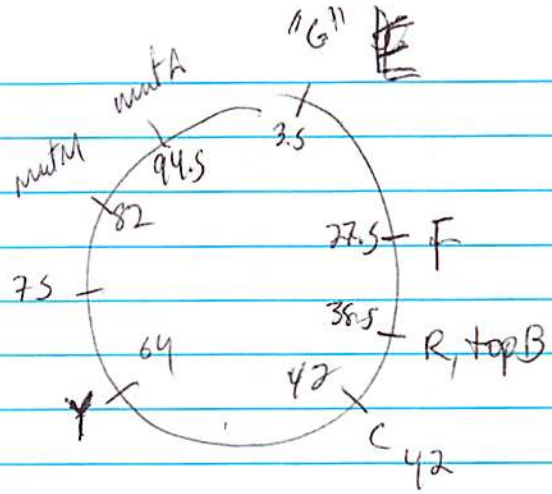
Reverse Chem. Mutagenesis
- use E.coli RTase
- produces C→T change

what is background for RTase

what about double hits?

Jeff Miller

Mutator Strains



mutM, Y } 8-oxy-G repair - removes A from A^{G^x}

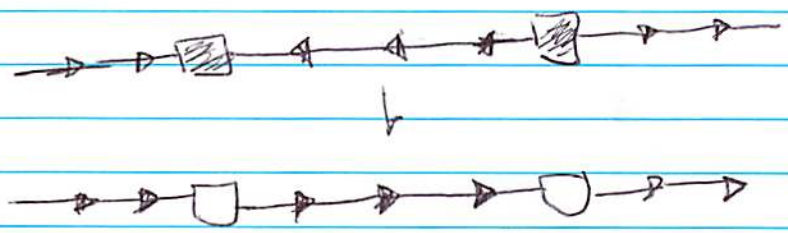
mutT - removes dG^xTP

mutA, mutC } $AT \rightarrow TA$; $AT \rightarrow CG$ } prevent transversions

mutF, mutG, topB } prevent rearrangements at short sequence homologies

a) lac⁻

① deletions of 17 bp duplication
② select for inversion in lac



③ duplication @ short repeats

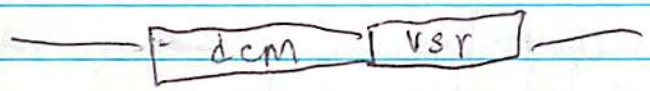
Mark Meuth - Colon Carcinoma

Is Microsat instab. related to flx?

Cell Lines expressing microsat. instability
① some v. variable
② some not
③ mutation accumulate during cell culture

- use this to measure rate
- can only detect >10% variation
- mutation rate ~ 1×10^{-3} for repeats

- also higher for HPRT pt. mutations
  - many are frameshifts
- no deletions
- selection



- transcribed from same promoter
- use deletion of these
- add vsr & dcm back from strain
- check mut. frequency

Active f(x) that may incr. deamination
- possibly cytosine methyltransferase

Suggest mutG is a control sequence which regulates expression of vsr.

Why regulate vsr?
① control restriction/modification
② control of G:A mismatch repair
③ limit mutations

lesions
↓ ↓ ↓ ↓
repair

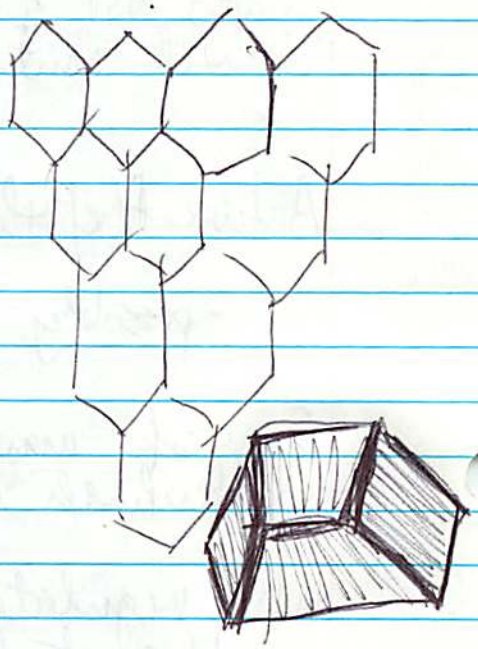
must separate (spatially or temporally) repair processes w/ overlapping specificities

Discussion

① what is contribution of metab. error vs. metab. damage to spont. mutation

② sequence context
- Scheraper

③



Posters

(1) Clarke
- ada - structure
- ogt -
- yeast
- human
- mouse
- Dat1 - B. subt
- AdaAB

- reacts best w/ 0.4 M NaCl

(2) Xiao
- M.Taq - yeast

(3) Sargentini

5% of WT γ-induced mutations are doubles. and most 2nd mutations are silent

Bryn bridges

- tyrA WU3610
  - no mutations accum. over time
- tyr+ accum. also when ~~selected~~ starved for leu, glue

D. Mosbaugh OSU

- ung DNA binding domain
- KVVIL.....SVR
- APH.....EQR
- ugi inh. b prevents assoc w/ DNA
- order doesn't matter
- ung peptides crosslinked to DTDP

Tomas Prolla

MSH2 MLH1 PMS1 interactions

Phil Hanawalt

Tx coupled repair

~~- why CPDs~~

Chris Selby

TRCF ① 130 KD
MFD
- interacts w/ stalled RNA pol
- assoc. w/ UVRA
- UVRB delivered
- normal NER
  - >100 copies per cell
  - deletion strain viable
    - same UV sens. as WW3610-45
    - no TxCR
    - grew normally

② - use ECORI block to tx.
- RNA pol. forms stable block
- Mfd allows for removal of RNA pol
- also removes RNA pol blocked by nucleotide depletion

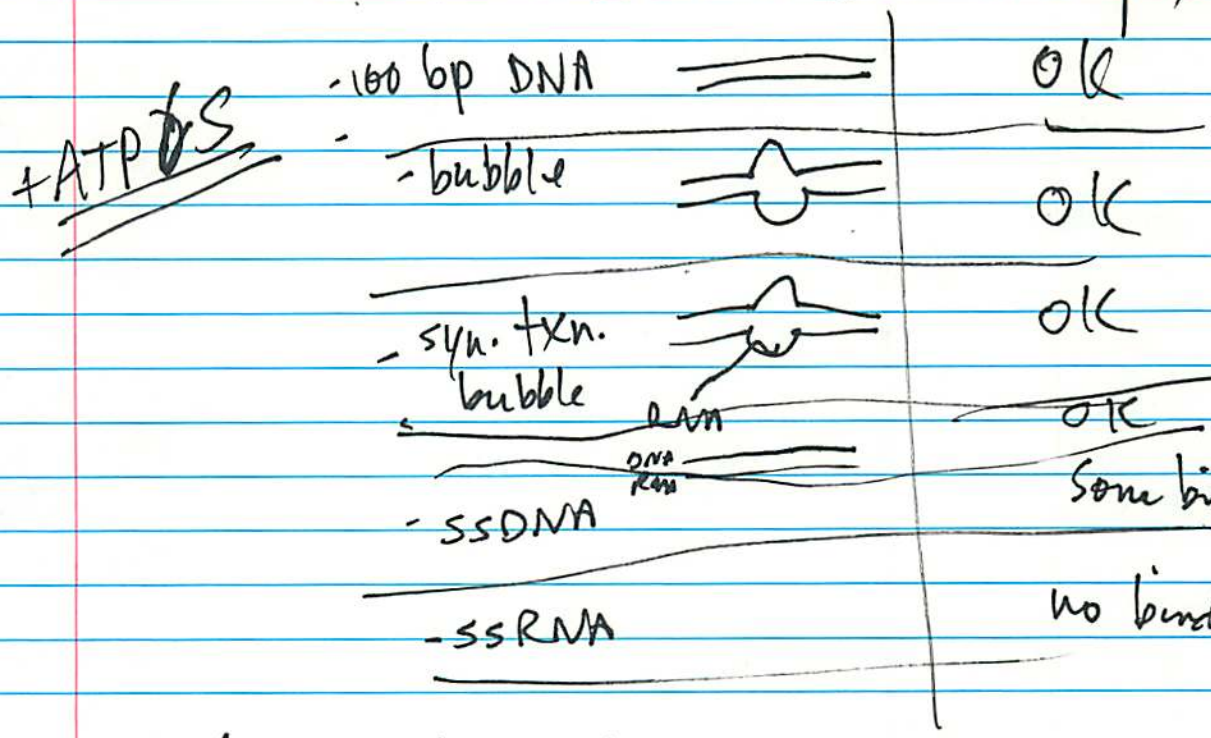
③ diff. deletion mutant
- 30% amino terminus deletion is proficient in removing RNA pol

UvrB region

② Trunc deletion --- Missing some of other end
- does not bind free RNA pol
- nor does it

③ TCR
- +15 - +11 is where TCR begins
- this is where RNA pol. conformation Δ occurs

④ think mfd recognizes some aspect of nucleic acid in stalled complex



⑤ binding observed only w/ those constructs w/ helicase motifs
- all binding needs ATPγS
- except one w/ pt. mutant in ATP hydrolysis region

④ hydrolysis of ATP needed for dissociation

⑤ HELICASE ACTIVITY
- more detectible for most
- checked w/ syn. txn bubble
- but no helicase activity
{maybe RNA is removed by sticking to RNA pol}

TCR in humans

- w/ D. Reinberg
- R. Drapkin

④ - Purified TFIIH - ERCC3 } in TFIIH
ERCC2 }

- complementation
* - could compl. XPC w/ something assoc. w/ ~~TFIIH~~

- TCR in vitro
- using ↑ in specific region
- block to txn.

iMellon

rRNA operons

WT ··· moderate strand bias
··· all rRNA repair is slower
···

MFD ·· reverse of strand bias
··· nts repaired more
··· even when reduce tx
·· tg repair repressed

Why ① diff RNA pol. complexes?
② tx rates?

rpoB mutants

- different tx termination
- assayed UV sensitivity

- Incr tx terminator
  - wt repair both strands
- Normal tx terminators
  - normal too
- Decr. tx terminators
  - tx strand OK
  - ntx decreased

Suggests the affect may be due to decr. tx. of repair genes.

Mismatch Repair

- mut H ... OK
- mut L } ts. repair like uts
- mut S }

Model

① mut L mut S bind around stall

② loop formed

③ MFD releases pol.

④ dimer in superc. looped DNA

⑤ enhances recognition by UVR A/B

3 Donohue

TCR & Pol II

① α-amanitin blocks TCR
② Ts pol II not TCR at non-perm. T°
③ pol I no TCR

TCR
= S II - TFII S
- allows RNA pol II to read through tx arrest
- nascent tx cleaved
- cleavage inhib. by α-amanitin

- ↑T blocks tx if on transc. strand
- S II causes ~~back~~ tx cleave at near dimer
- phr does not recognize CPD even w/ tx cleavage

- TFIIH/IID/IIE ... fraction D
  - no detect. effect

Tony Leadon

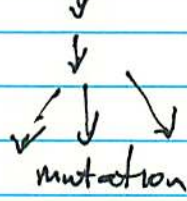
P. Cooper

- ionization also damage tx coupled repair
  ↳ defective in CSA

R. Fuchs - Lesion Structure & Mutagenesis

Goal: Predict type & frequency of mutation from lesion

① lesion



② Structure ...

- ss vs ds DNA
- ③ mimic replication intermediates

④ genetic vs. biochemical screen
- characterize all including silent is important

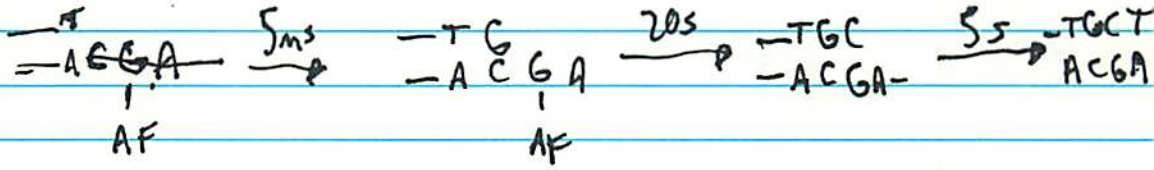
⑤ adduct size important
  small
  bulky

⑥ adduct site important
- coding vs. non-coding

suggest TCR may reduce mutations
bec. lesions in both strands worse
than those in one

Bioch 33:764 ... Translesion syn. at AF / AAF

T7 pol



AAF - slows down even more

Sequence Context

- AAF hot spots in repd. seqs ≠ NarI

Strand Coherence

Leading vs. Lagging Strand
① diff. errors of pols
② diff. freq. of translesion synth

C. Lawrence; Mutag - Properties of UV photoproducts

Parameters determining mutagenesis

SS vectors TT-6-4

TT	66%
TC	18%
TA	3%
TG	3%
GC	8%

Abasic site in yeast

C	64%	18
A	10%	10
G	0%	0
T	0%	0

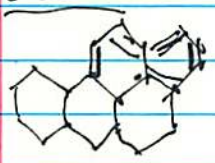
6-4 TC UVB isomer

- 1st nucleotide inserted influences 2nd

- rev3
  - DNA polymerase motifs
  - inessential
  - out of frame ATG reduces $t_{1/2}$ 10-100x
  - overproduction not toxic

Ed Lockler

LM



BPDE

plasmid → supF → screen for Mutants in Ecoli ... almost all genotypic are phenotypic

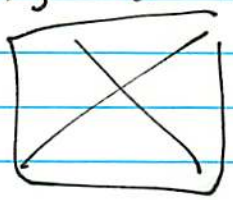
80°C x 10'

Guanine mutations

① nearest neighbor

5'	3'
T	G
A	G
T	G
C	G

G		
↙	↓	↘ ±
T	A	C
13	0	1



– roughly equal

- heat + heat not many differences
- except at one hotspot

- suggests this may be due to AP sites.
- but

Hotspot

- made site specific lesions
- similar to random pattern

Semi-targeted mutagenesis

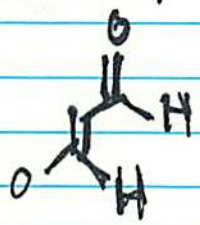
- adduct at 115 induces mutations at 116 as well as 115

- more at 116 w/ 115 adduct than 116 w/ 116 adduct
- no affect of SOS

L. Marnett

OH → DNA
OH → prot.
OH → F.A. → Malonaldehyde --- mutagenic
F.A. → 4-hydroxynonenal

1-04 Malonaldehyde

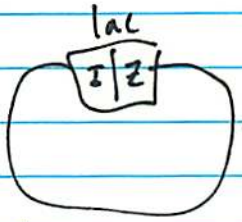


+ dG → M.G major in vitro product
+ dA → M.A
+ dC → M.C

- use mass spect. to detect adducts
- counted adducts:
  1. none in testes
  2. 100x > in liver
  3. none in S. typh.

Random Mutagenesis

- ssM13



+ MOA → select lacZ mutants

- SOS system required
- mutations at/@

@G· 92% G→T

@A 100% A→G

@C 88% C→T

- -vectors

- mutation freq incr. in uvrA

Art. Grollman

① Diff. DNA polymerase

β--

② Seq. context
- Biochem 32:7531 Shibutani et al

- How far do you have to go for sequence context affects

- Mike Simon

- contacts w/ DNA pol are important
- sequence Δ's may Δ mech. of mutation

M Goodman
- base stacking important
- Cations important
- H_2O important

A Rule?
- not in mammals

p53 spectrum

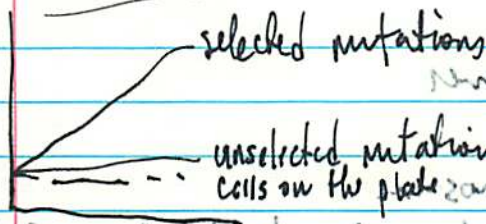
Complexities of in vivo
① screen
② selected
- may vary among & w/in tissues

③ ¼ of 3000 mutations are transitions at CpG's.
@ varies among tumors

ftp from embl ... excel spreadsheet

④ seq. heterog. among species makes it diff. to determine seq. context

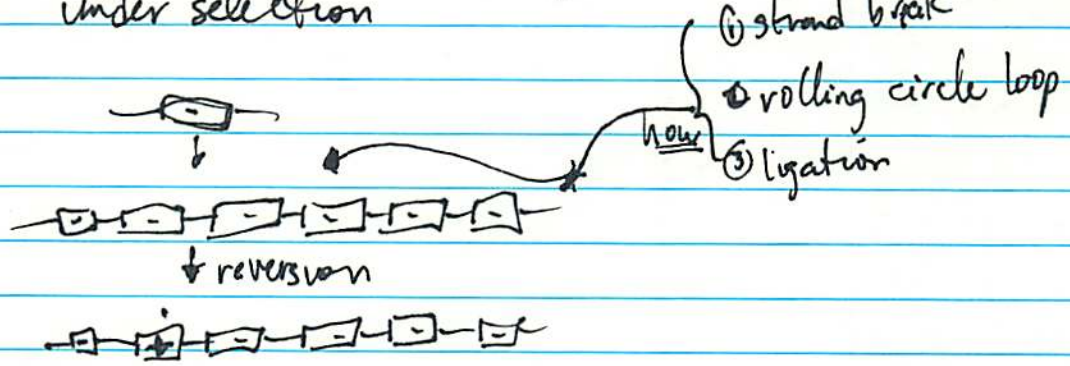
John Roth



Model

1. most of mutations are leaky
2. duplications - are common
3. strong selection leads to large arrays of tandem repeats
4. amplification of mutant allele could lead to survival / growth
5. growth
6. tandem arrays disappear
7. gives appearance of mutability to base pair under selection

but about recreating w/ multiple



what about IS10's

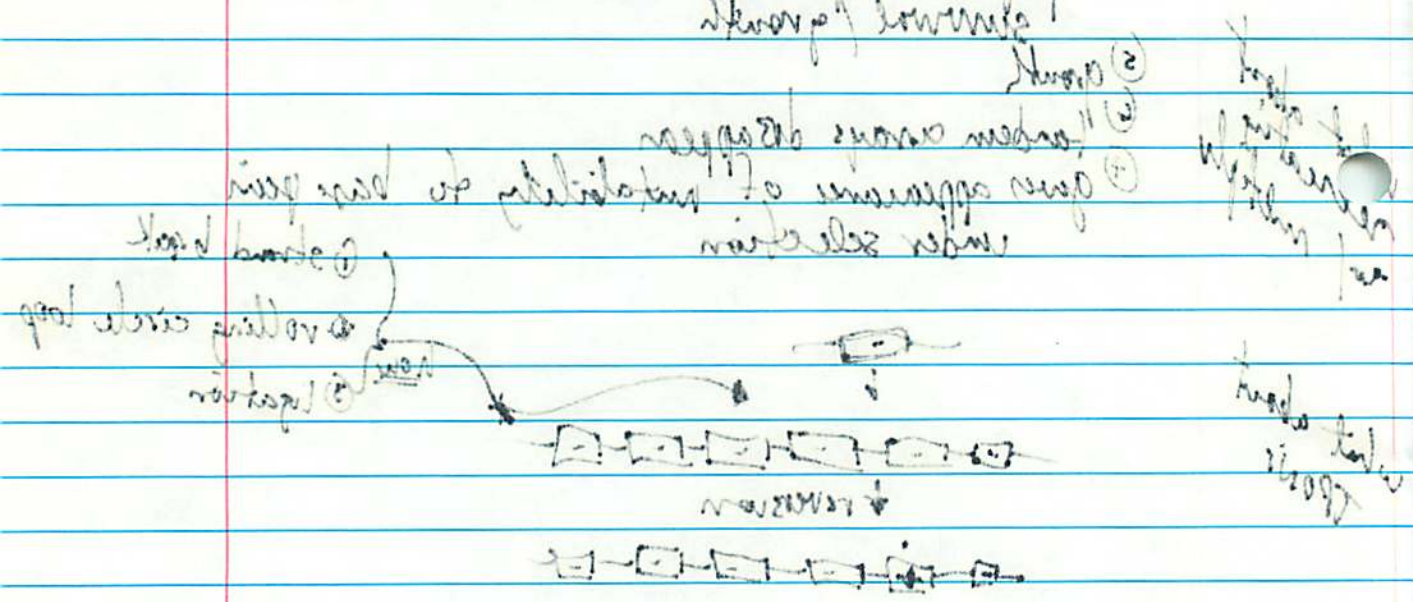
Data

- lact mutations
- some "adaptive"
- some flat
- strong correlation w/ leakiness (incl. Tn10 insertion)

- Growth on plates
- Not much rec. dependence

① Mutations in F vs. chromosome
- suggest may be due to duplication of F

② sectoring of colonies



(6) Kunkel et al Error Prone - Replication

- thioredoxin confers high processivity to T7 DNA pol

(24) Taccioli et al Ku80 = XRCC5

(3) L3 deamination of CPD's

(4) Barlow et al.
- umuD' binds DNA in presence recA
- umuD'C inhib. repair of DNA damage by recomb.
- A1730 (deficient in IM) is dominant in 505

(5) 1 GFRGEAL hPMS1 hPMS2

370 VDVNVHPD K
T A T

3) Selva et al

- homeologous rec 100x < homologous
- homeology incr. in PMS1 mutants
  MSH2
  MSH3

Singh
RPA appears to regulate expression

XPG mutations

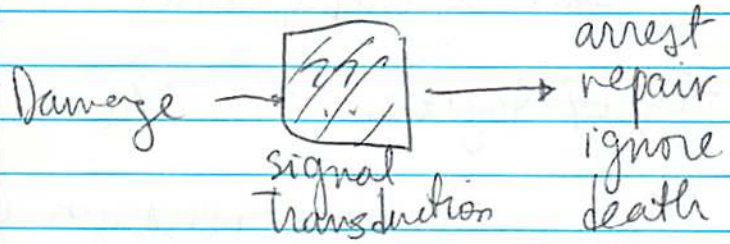
Mutational Origins & Genetic Disease

① G x E interaction
② Signal transduction

Genomic Integrity

① repair
② proofread
③ chromatin st(x)
④ chromosome st(x)
⑤ mitotic apparatus

} signal transduction pathways to scan for these



Cancer origins / Genomic Instability

① transformed cells
- viral transformed have amplification
- is it step wise or instantaneous

② don't respond to things like aPACA and stay through cell cycle

③ human papilloma viruses
① high risk types ... lead to malignancy
② low risk types .... not as likely

what if reverse transformation

① cells transformed have ↓ p53

② transformed cells normal phenotype until challenged w/ cell cycle inhib or damage

④ check for PALA resistance (usually caused by amplification)

- 100% of ~~PALA resist.~~ clones came by amplification

- E6/E7 together - 100% amplify

- high risk { - E6 } imm. upon expression checkpt. control is lost
- E7 }

Cell Cycle Complexes

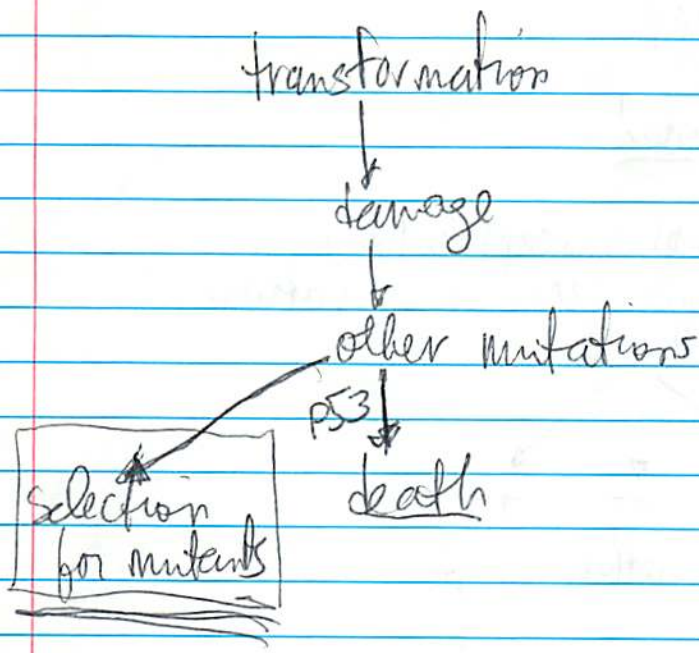
① normal cells — cyclin, PCNA, cdk1/2, p21] suggests that these two are ~~that~~ some of the molecules that respond to ~~environ~~ regul

- these cells are mortal { ② transformed cells are missing PCNA & p21

③ similar pattern w/ cyclin A, cyclin B1

④ not seen w/ low risk - viruses

but any changes in ratio

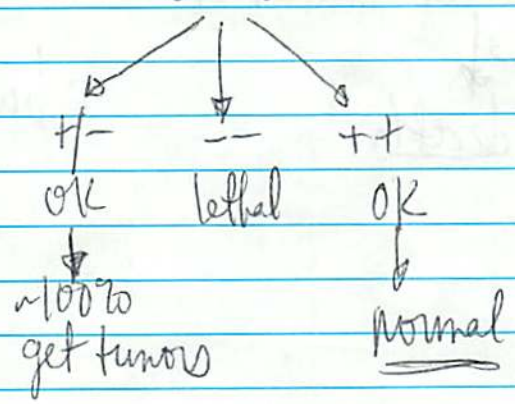


- ubiquitination is involved in p53 degradation but not with these cyclins
- but what about RAD6?

Cheryl Walker

Eker Rat Model

- tumor susceptibility
- single germline mutation



Carcinogen Susceptibility

- Normal rats -- v. rare tumors
- Eker Rat -- lots of tumors
  -- test whether get even more w/ carcinogen?

DMN →

Yes!

Why tissue specific

1. mutation freq.
2. determined by cell type

↓ selection

In vitro vs. in vivo.

- transformed colonies vs. tumors

Retroviral tags

① heterozygotes
↓
② retroviral infection
↓
③ look for Δ in transformation

② retro. infection cause more Δ in transformation in heterozygotes

Carl Anderson - DNA Activated PK

DNA-PK
- Ku (p70 (p80) ~~XRCC5~~
- PrKdc (~450KD)

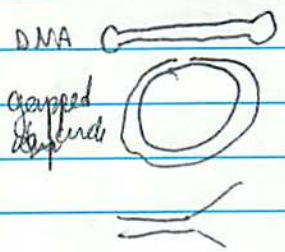
TIBS review

Regulates Tx — Phosphorylates these

TOPO I	p53	Jun	SRF	Oct-1	TFIID
	Sp1	Fos	Myc	Pol-CTD	HSP90

Phosphorylates RPA

Ku activated by

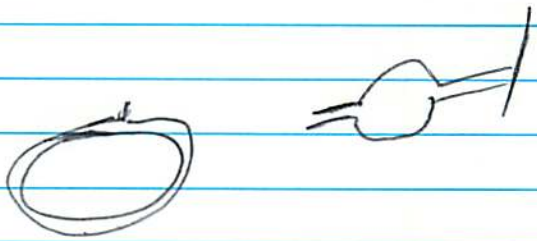


not activated

- suggests some of these may be phospho. because in the assay they get concentrated near DNA

Phosphorylation sites @ P53

- SIS



ADAPTIVE MUTAGEN. IN A STAT. PHASE AUDIENCE

I. Marnett GK ✓

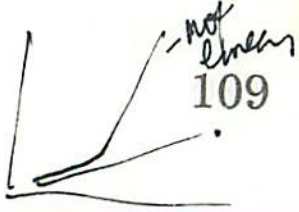
Multicellularity

— Death is a viable option - Rhizobium

- RNA assoc. mechanisms

P. Foster

Pat showed plot of Lenski mutation : ? growth



① mutations in non-lethal selection conditions
② stationary phase conditions

How might genetic variation be generated in non-dividing cells

① Evidence
  ⓐ L+D fluctuation test
  ⓑ time

cell turnover still get L+D
- poisson distr. can come from other things

Turnover calculation
- use normal exponential rate

How much have to change expectation of mut. freq. to get data.

No change in viable count
- 2x increase
-
- cannot get linear incr. from growing population
  - but what about turnover

② Evidence for adaptive
  ⓐ don't accum. under non-spec. stress
  ⓑ not accum. mut. to 2nd phenotype
    - says it's not that hard to do
    - uses v.f.R

says not entering hypermutable state but

③ Mechanisms
- tx not mech. bec. no accum. in absence of selection

FC40

- Recomb. of rect. in equatorial
- over time most -1 frames
- most in runs

Stinks-Robertson- Adaptive

lys2 ΔBgl
- reversions are intragenic
- most are 2nd site

lys⁻
↓
grow ind. in rich media
↓
plate w/o lys

- no correlation among early & late
- why distribution not useful
  ① lys⁻ continue to divide
  ② time of appearance related to growth rate
  ③ delayed due to phenotypic lag

- Do they divide
  - carrying kills growing cells
  - what about replacement
NO - what about carrying affect weak in selection

Growth Rates:
- reconstruction
- how long lys+ colonies appear

Phenotypic lag
non-reverting lys⁻ cells
↓
transform w/ lys2 plasmid
↓
plate
↓
all at same time

what about phenotypic lag of revertants

Are they specific

① starvation not mutagenic

Time Dep. Recombination

-accumulate even w/ other starvation

- what about non-coding lesion

more lacZ

DNA rep

copy num

Tx- P gal

- use isogenic +/- ~~promoter~~ neg. regulator
- much more w/ higher Tx
- not due to +/- regulator (look at other genes)
- rate prop to Tx

Why

Prot. Compensatory Changes

Prot. Engin 7:349 7:341
PNAS 91:98

Bryn Bridges
- mfd- ⟨ no directed mutation
- but mfd will dying

- catF mutants die on plates; esp. w/ high T°
  - bubble w/ H_2O_2

SAM - starvation assoc. mutation
- specificity is different
- neutral

ADAPTIVE
-

DIRECTED
- Fitch predicted this

GENETIC TOXICOLOGY
GORDON RESEARCH CONFERENCE
Colby Sawyer, July 26-30, 1993
Sid Aaron, Chairperson; Julian Preston, Vice Chairperson

Monday Morning Session Chair: Tom Kunkel "What is the nature of 'spontaneous' mutagenesis?"

1. Speaker: Roel M. Schaaper
   Title: DNA replication errors and spontaneous mutagenesis in *Escherichia coli*.
2. Speaker: Leona D. Samson
   Title: Endogenous alkylation-induced mutagenesis in prokaryotes and eukaryotes.
3. Speaker: Patricia L. Foster
   Title: Mechanisms of Adaptive Mutation in *Escherichia coli*.

Discussion Leader: Bernard G. Strauss

Monday Evening Session Chair: James Crow "What is the impact of mutation in populations?"

1. Speaker: Joni Drost
   Title: The number of cell divisions ancestral to male and female gametes in Drosophila mouse and human.
2. Speaker: David Houle
   Title: Total rate of mutation of deleterious genes affecting fitness in Drosophila.
3. Speaker: Carter Denniston
   Title: Mutation component of genetic disease, with special attention to threshold traits.

Discussion Leader: Brian Charlesworth

Tuesday Morning Session Chair: Tom Skopek "What are mutational spectra trying to tell us?"

1. Speaker: Barry Working
   Title: *Hprt* mutational spectra in human populations.
2. Speaker: Bernie Kunz
   Title: Mutational specificity in a yeast tRNA gene: strand bias and strand identity.
3. Speaker: Veronica Maher
   Title: Insights into mechanisms of mutagenesis obtained from mutational spectra.

Discussion Leader: Ken Tindall

Tuesday Evening Session Chair: Tom Cebula "New strategy for rapid detection of mutation."

1. Speaker: Peter A. Carutti
   Title: RFLP-PCR Analysis of *ras* and *p53*.
2. Speaker: Vilhelm A. Bohr
   Title: Genotoxic damage and repair.

Discussion Leader: Eric Eisenstadt

Wednesday Morning Session Chair: Ray Tennant "Evolving transgenic systems for study of germ line mutagenesis."

1. Speaker: Rick Woychik
   Title: Insertional mutagenesis and the molecular analysis of developmental mutations.
2. Speaker: John Schimenti
   Title: A recombination-based transgenic mouse system for evaluation of genotoxicity.

Discussion Leader: Robert Langenback

Wednesday Evening Session Chair: Dick Albertini "Human genetic disorders and mutation."

1. Speaker: Steve S. Sommer
   Title: Assessing the underlying pattern of human germline mutations: Lessons from the factor IX gene.
2. Speaker: David W. Yandell
   Title: Comparison of the germline versus somatic mutation spectra in RB and P53 tumor suppressor genes.

Discussion Leader: Bruce Kovacs

Thursday Morning Session Chair: Marshall Anderson "What role does mutagenesis play in carcinogenesis?"

1. Speaker: Helmut Zarbl
   Title: NMU induced rat mammary tumors arise from cells with pre-existing Ha-ras-1 gene mutations: implications for mechanism of carcinogenesis.
2. Speaker: Roger Wiseman
   Title: Analysis of p53 mutations in tumors of humans and experimental rodents.

Discussion Leader: Edward Bresnick

Thursday Evening Special Lecture: Tom Caskey "Unstable repeat sequences as a cause of disease mutations in man."

Friday Morning Session Chair: Bill Lee "Is there any germline risk?"

1. Speaker: Ken W. Turteltaub
   Title: DNA Adduct Dosimetry: New Methods Offering Increased Sensitivity.
2. Speaker: Harvey Mohrenweiser
   Title: Role of Sequence Specific Gene Mutations and Non-Traditional Inheritance in Estimation of Germinal Mutation Rates.

Discussion Leader: John Ashby

Gordon Conference Schedule:

Sunday Evening:

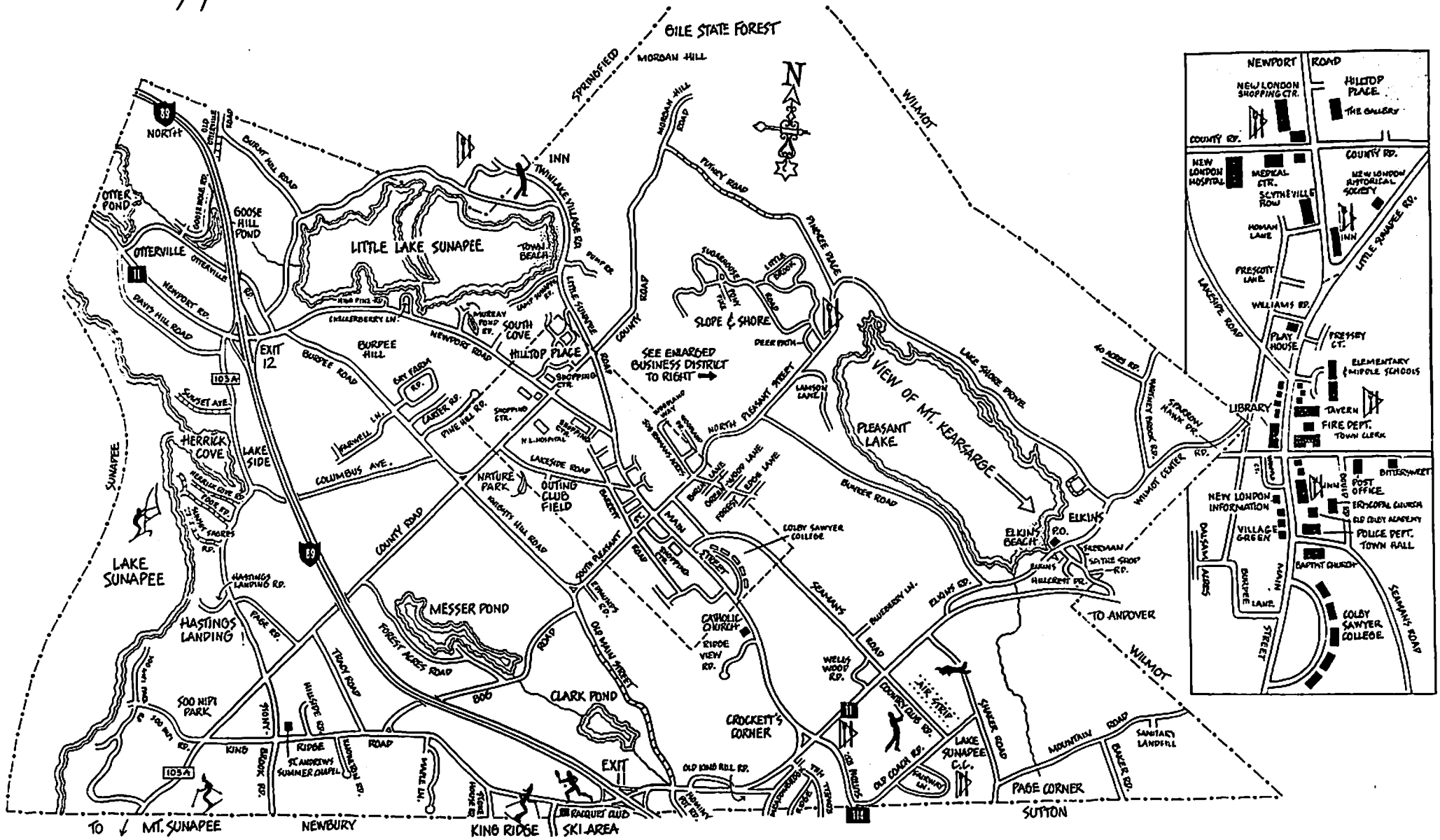
Bus arrives/registration/dinner/reception after dinner

Monday/Thursday:
9:00-12:30
Formal sessions with a coffee break mid morning. Photo on Monday morning!
12:30 (prompt)
-Lunch
Afternoons
-Free of all formal activity*
4:30-6:00
-Poster sessions (as quantity dictate)
6:00
-Dinner
7:30-9:00
Formal sessions

Friday
9:00-12:00 (note shorter time!)
-Formal sessions
12:00
-Lunch
1:00
-Bus departs

*** Wednesday afternoon there may be a canoe trip if sufficient interest is shown.**

Welcome to NEW LONDON, NEW HAMPSHIRE



Map Courtesy of: Johnson & Dix Fuel Corp

NEW HAMPSHIRE

Quality

IN NEW LONDON