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: Larry Wolfing
   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. Cerutti
   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 Langenbach

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:

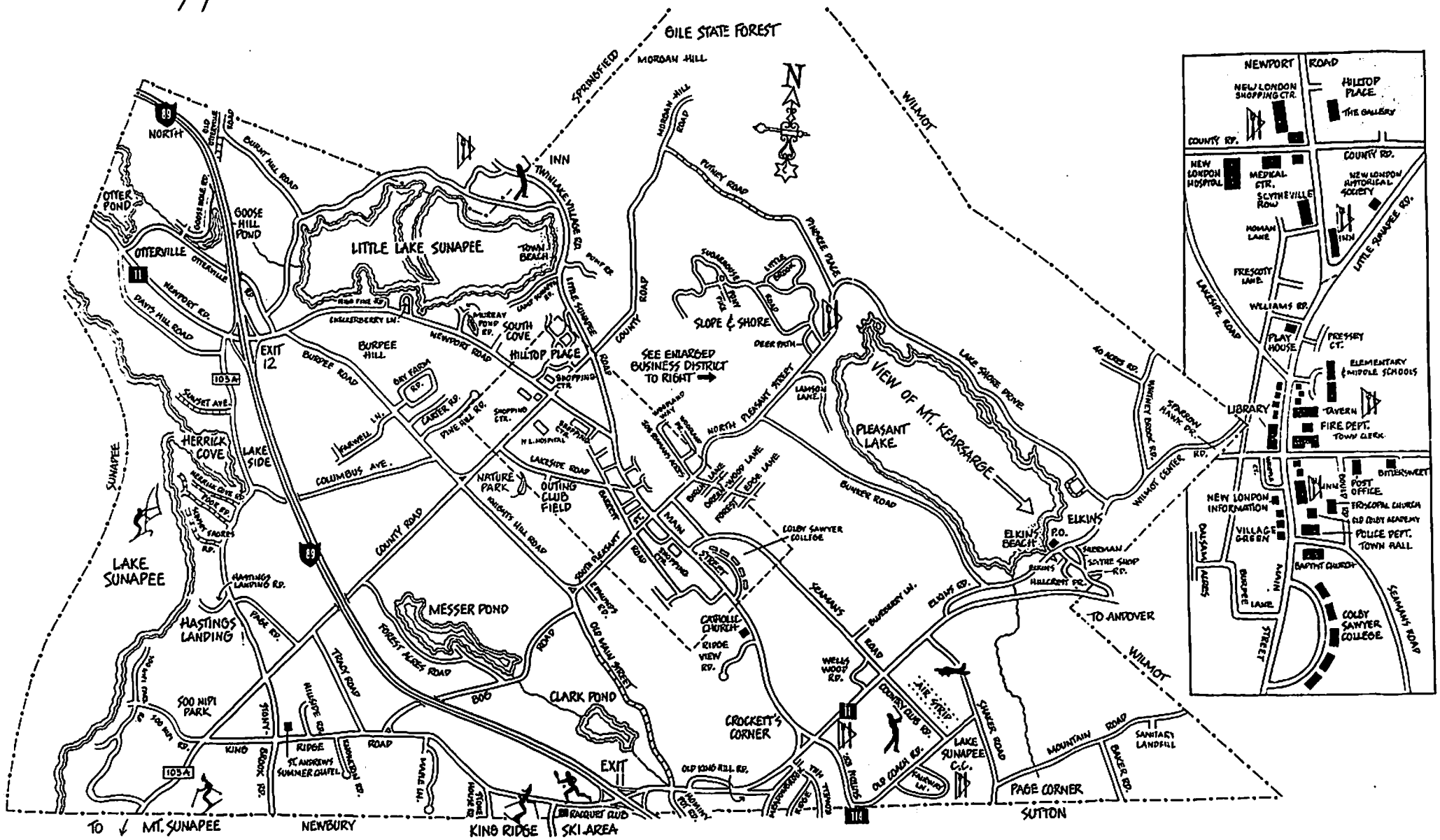
Bus arrives/registration/dinner/reception after dinner

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

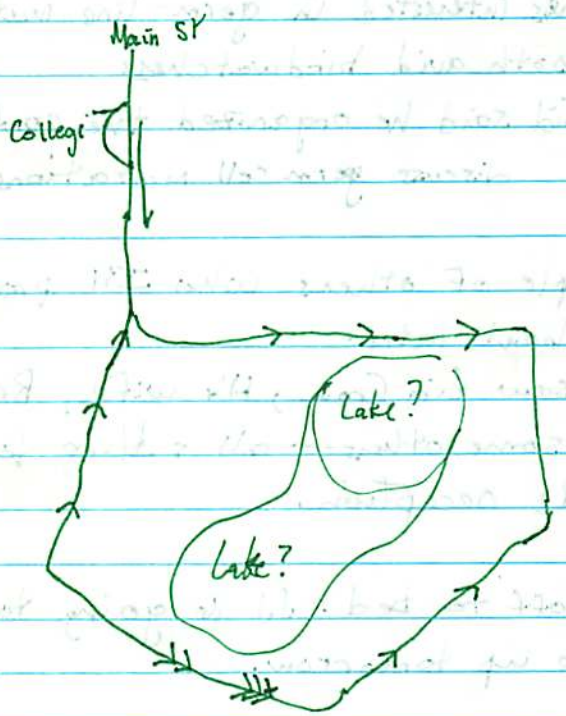


Map Courtesy of: Johnson & Dix Fuel Corp

NEW HAMPSHIRE Quality **IN NEW LONDON**

9/25/93

-Arrived at Colby Sawyer College @ ~4 pm
-Unloaded stuff
-Got my bike ready & went for a ride



-about 15 miles

-Got back, took a shower, & off to dinner

-Dinner
- met lots (well some) people
-SJ - from Bueno Aires
-now at U-Mass Worcester
-works on Yeast Mismatch Repair

675

Sid Aaron & His wife Judy Mayo
- both very nice
- she was/is quite a story teller talking about trips to India, Indonesia...
- they're both from Upjohn Co, Kalamazoo, MI
- he's interested in germ-line mutation risks
- both avid birdwatchers
- Sid said he organized the conference to discuss germ cell mutations

A couple of others who I'll probaby write about later.

Also- saw Jim Crow, His wife, Brian Charlesworth & some others... all sitting together at the reception.

well- off to bed. JJ is going to wake me up tomorrow.

Roel M. Schaaper

†Kunkel · Spont. Mutation

Types
- replic. errors
- damage T. Lindahl Nature 362:709
  - deamination 100x > in SS
  - oxidation of G
  - depurination
  - methylation
  - dNTP damage e.g. 8-O-dGTP (pairs w/ A)

Mechanisms
Replication
- strand slippage, proofreading, mismatch
- Asymmetry
  - leading/lagging strand
  - architecture
  - dNTP pool
  - sequence
  - timing
- other enzymes
  - topos, methylases...
- adaptive mutation

Roel Schaaper - DNA Replication Error & Spont. Mutations in E coli

E.coli Spont. Mutations in LacI

- 414 Mutation in 200 BP of DNA binding domain
  - 70% substitutions
  - this % changes if you look at other regions
  - suggests hotspots due to many factors

- Sources
  - replication errors
  - damage
  - repair
  - transposons

- Antimutators provide useful info
  - ① compare mutation rates & determin what antimutator does
  - ② or isolate mutants w/ "better" certain steps like replication & then look at mutatio

- Replication
  - polIII dimer (22 subunits)
  - only two subunits very important
    - α - DNA E - polymerase 10^{-5}
    - ε - DNA Q - proofreader 10^{-2}

Mismatch
- mut H, L, S - 10^{-3}

① start w/ mismatch mutants
- isolate suppressors

② use papillation assay
- papillae are mutants (gal K)
  have advantage

③ mutagenize coli

④ scan for fewer papillae (or more)

ANTI-MUTATORS DNA E - 7 antimutators

MUTATORS DNA Q - all in DNA E ∴ easier to make polym. better

- many mutators
- only one in DNA E

OTHER GENES ⑤ checked strains for other mutation
- rif^S → rif^R
- antimutators are lower

⑥ transfer to w.t.
- ~~DNA~~ dnaE911 - ~two fold ↓ in spont. mutations
- but may reduce other types of mutations

⑦ UV mutagenesis
- not much change
- not much in SOS in cp+^{C}

How does it make fewer errors?

13 cases
11 different a.a. changes
- spread out over 400 aa.
don't map into conserved domains
which are activis sites

How might incr. fidelity?
① improved selectivity
② improved interaction w/ proof. subunit
③. may be impaired, slow polymerases

see Tilly

THIS MAY BE WHY MORE ANTI-MUTATORS THAN MUTATORS IN POLYMERASE

- but doesn't explain specificity

Combined these mutations w/ other mutations in polymerases? (proofreading mutants)

dnaE911 mut D5 - looked at mutations in lacZ
- got big changes in certain mutations

but may not be completely defectivi in proofrea

Specificity
- galK mutation was one type
- try isolating anti-mutators of other mutations

WHAT ABOUT SELECTING FOR LOSS OF W.T.

Other pathways
- looking for anti-mutations in other pathways

??

B. & Tilly - suggests that these strains do not do much bypass ... and thus mutations don't show up

Fred Hutchinson - asked about polI anti-mutators because of the X-tal structure

Pat Foster - mechanisms of Adaptive Mutations in E. coli

Two aspects
① They do occur in non-growing non-replicating
  ⓐ deviations from L & D
  ⓑ mutants contain & accumulate w/ time

Deviations
- says nothing can explain incr. in Poisson nature over time

How know not growing?

"Turnover required"
- if mutations normal

Plugs - plated for viability

Lenski double mutation

BUT WHAT IF HIGHER RATE OF REPLICATION ERRORS - what about

WHAT ABOUT REPLICATION W/O DIVISION

A.

How know mutants adapting ↑ delay overlay
① not induced by starvation
② mutants do not accum when not beneficial

New other mutations
① Rif^R - doesn't accumulate

Alkylation damage

David Stadler - Spectrum of Spont. Mutations in Neurospora

1) want to detect most/all of mutations

2) GENE: mtr
- neutral a.a. permease
- uptake of neutral aa & analogues
- select for resistance to analogues
- can also select for reversions

3) TWO WAY SELECTION

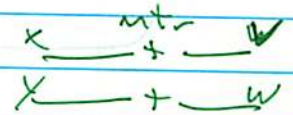
	MIN	trp	anthranillate (in trp pathway)	aut + FPA (inhib. for mtr not needed but mtr)	
trp-2	0	+	+	0	} growth
trp2 mtr	0	0	+	+	

trp2 → select on FPA → grow = mtr⁻

grow = mtr⁺ ← select for trp growth

4) GERM LINE
- SPECTRUM?
- DOES SEXUAL CYCLE MODIFY MUTANTS?

- ISOLATED USING CROSS
- HARVESTED ASCOSPORES
- & DID ABOVE SELECTION



SOMA

	#	
LARGE DELETION (flanking lost)	6	
DELETION (Revers. lethal)	3	7
BASE SUBST.	67	12
FRAME SHIFT	4	5
SMALL DELET.	32	
DUPLIC. (3-950 BP)	9	17
LEAKY	10	
?	4	
RIP		
TOTAL		52

- large ones reverted w/ highest %
- grow on both media

- even though no duplication deleted

- some minor hotspots

SIGNIFICANT DIFF. IN SOMATIC & GERM MUTATIONS.

NO EVIDENCE FOR RECOMB. MUTATION
- not occurring in meiosis
- not biased to occurring in X-overed strains

- Some of the deletion/dup. hotspots are at 2ary structure

Discussion

MULTIPE MUTATIONS IN TUMORS

① MCB 11:3163
" 12:767 ?
p53 CANC RES 53:1162
" 52:1695
" 51:6194
Lancet 339:576

} sees this in in vitro error prone polymerase: "stuttering"

what about transient mutators?

Eric Eisenstadt
- suggests no generalities can be made about spont. mutations

- but can make for UV

- IF YOU COMPARE MUTATIONS IN HPRT TO OTHERS NO HIGHER, BUT DOUBLE MUTANTS OCCUR AT EXTRA FREQUENCY.

- SHUTTLE VECTORS IN MICE

	PTS	DELET
TWO STRAINS -	80%	20%
	20%	80%

JGlervine RMSchaaper DMDeMarini

① complex frameshifts generated by pKM101 in S. typhimurium

- hisD 3052 - 2 base deletion induced by 4-AB
- w/ plasmid - only 85%/15% complex
- distance between repeats affects frequency

JMCahill AEMcElroy -
Benzpyrene adducts in flounder

① new chromatog
② large inter-indiv. variation

WHKoch EHErikson TACebula

pKM101 (MucC) plasmid

low dose MMS - mostly transversions
higher doses - mostly transitions

- since mutagens cause diff lesions dose may affect mutation spectra
- also seen in BP HGPRT.

JJMicet BoParker RSLahue
- New mismatch repair
- MSH4? may correct insertion/deletions

mutHLS - in E. coli fixes up to 3 bp.

- see Reenan & Kolodner Genetics 132: 963

HLRoss JEPagel AHBiggen AD.ppes
7-BrMeBa induced in supF

- sites of polym arrest do not correlate w/ hotspots

Biochem 32:5526 SSSastry HPSpalding QSHoang APNPhillips ASancar, JEHearst

Laser induced protein-DNA X-links via psoralen furansid, monoadducts

-366 nm

WXiao LSamson
In vivo evidence for endog. DNA alkyl. damage as a source of spont. mutations in Ent cells

- MTase - mention MGT1-O6-methylguanin DNA methyltransf
- 3MeA -

Yeast - spont. O-alkyl damage
- MTase, 3M eglycos, AP endo repair
- 3MeA removes lesion & converts to AP mutagenic site

SAYS NO REPAIR IN DROS. SPERM
MOUSE " "

Mutations in Populations

Germ Line # of cell divisions ancestral to male and female gametes

Germ Line
Drosoph ♀

fertilized egg → DNA replic → nuclei

Drosoph

	♀	♂
Divisions	16 before pupation	14 before
	13-18 stem cells	17 stem
	5 oogenesis	5 spermato
	34-39/generation	36/generation

IS THERE ANY DIFF IN AGE AT REPRO

Mouse ♀ ♂
25/generation

Human 31/generation 39?/generation

	human ♂	human ♀	Mouse ♂	Mouse ♀	drosop ♂	drosop ♀
- aver gener.	30	25	0.75	0.54	0.049	0.068
- # division	?29	31	62	25	36	36
- divis/year	13.3	1.24				

Mutation Rate

Male Driven Mol. Evolution
Miyata et al 1987, 1990

Factors affecting sex differences

① mutations before germ cell differentiation

② methylcytosine
- germ line in mammals - only male methylated

Jim Crow

$$\mu_x = \mu_p + \delta\mu(x - x_p)$$

x = age
x_p = age puberty (13)
$\delta\mu$ = increase in μ/year

- mutation problem caused by old men

- mutation rate incr. w/ # of cell divisions in male (more than linear)

- some genes show paternal age effect
- some don't

Two alternatives
- ① mutations rare w/ big affect (variance ↑)
- ② mutations common w/ little affect (mean ↓)

$\Delta M, \Delta V$

- Fitness affected v. much
- Most mutations affect lots of things

Variance due to mutation v. high
Positive correlations among life history traits

what about multiple mutations?

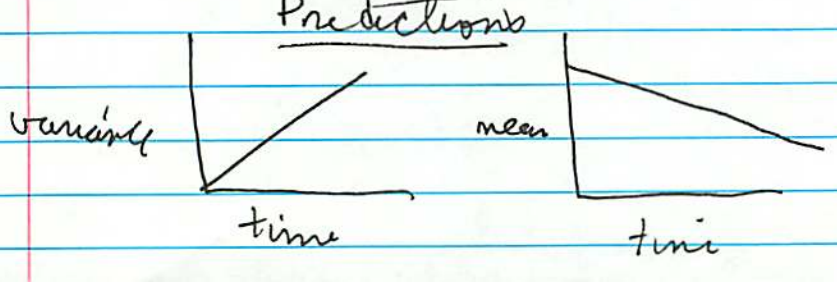
David Houle

Mutation rates
- D. melanogaster
  - allow spont. mutations to accum. w/o selection
  - measure phenotypic consequences
  - limit recombination

Limit recombination w/ balancers & using males
Limit/eliminate selection
- used strain lives
- isolated chromos
- copied it
- Cross
  - take only 1 male
    (∴ drift high)

Assay - homozygotes
- early fecundity (days 5-6)
- late fecundity (days 27-28)
- female long.
- male long.
- size
- "fitness" - $\frac{+i}{bw^v}$ vs $\frac{+i}{ri}$
  from gene frequency equilibrium

Predictions



Carter Dennington

Estimate of effect of raising mutation rate on human populations

B = Burden u = mut rate

Induced Burden = Spont Burden × Relative Mut. Risk × Mutation Component

$B \left[\frac{\Delta u}{u}\right] \frac{\Delta B/B}{\Delta u/u}$

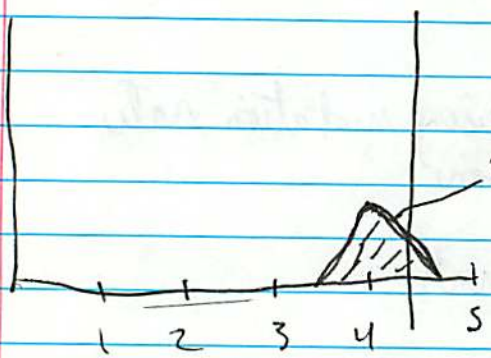
$\left[\frac{\Delta u}{u}\right]$ ← estimated from other species

$\frac{\Delta B/B}{\Delta u/u}$ = relative change in burden compared to change in mutation rate

Threshold Traits

liability (x) ∝ Genet & Envm.

Variance in trait has diff. (lower) heritability than the liability



liability = presence of mutant genes

B. Charlesworth
- mutation rate in D. melano v. high
- w/ low selection & small populations genomes should "go to hell" v. fast by mutation eg non-ps cpsts
- because 50% of deleterious affects are due to small effects then gene therapy can only work on other 50%
- use neutral genes

WHAT ARE MUTATION SPECTRA TRYING TO TELL US

MUTATION SPECTRA - THE PATTERN OF MUTATION THAT AGENT LAYS DOWN IN A PARTICULAR GENE

SPECIFICITY

Chemical exposure → adducts → repair → replicated
↓ mutation
↓ selection

HARRY VRIELING - HPRT in humans

T LYMPHOCYTES → PLATING → MUTANTS → cDNA sequencing

FINGERPRINT OF EXPOSURE
- use many exposed indiv. to remove background

TWO GROUPS
- SMOKERS - DISTRIBUTION not much different from controls
- LESCH-NAYAN - also similar to controls (base change)
- ETHYLENE OXIDE EXPOSURE - also v. similar in spectra, types

WHAT BP IS CONA, sequencing valid?

∴ MUTATION SPECTRUM IN HPRT IN HUMANS IS DIFFUSE.

DIFF IN SPECTRA - WHY?
(1) dose dependency
① kind of mutations that can be recovered
② extent of repair processes
③ amount of time betw induction & fixation

DISTRIBUTION ACROSS GENE
① SMOKERS = CONTROL
② EO = ONE HOTSPOT

CHECKING TO SHOW CAUSATION OF HOTSPOT

① RATS
MNU - induced mostly GC→AT
ENU - mostly AT→TA

WHY? - suggest diff. in damage induced

MNU SPECTRA
- most species GC→AT
- Drosophila - not. SUGGESTS DUE TO METHOD OF IRRAD MALES.

ENU SPECTRA
- many much variability

UV SPECTRA
CHO-9 - WT all types of changes, most on NTS (1:8)
- UV sens - most are GC→AT, most on TS (7:1)
↓
CHO mutants (3 lines)

EMS
CHO-9 WT: GC →(most) AT some AT→GC
EMS-cll mutant GC →(most) AT rest deletions - XRC-1 mutant

THINKS CAUSE
- transcription by pol III on plasmid

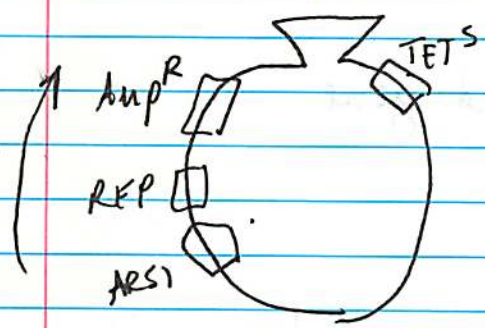
YCpMP2
pol III
pol II } ∴ pol II could remove NTS damage

Would/if you expect you to have to shift-

YCpJA1
III
II } ∴ pol II would remove TS

LEADING VS LAGGING STRANDS
- lagging might disengage & allow error free to work late ∴ less mutation

prob. this fork



∴ YCpMP2 - pol III lagging strand & txs same

YCpJA1 = pol III leading & txs same

LEADING VS LAGGING
① may affect replication errors

Bernie Kung yeast tRNA - strand bias / strand identity

SEQUENCE CONTEXT
① production of replication errors / damage
② correction / repair
③ efficiency of translesion synthesis

GENE CONTEXT
① chromos. position
② orientation

∴ GENE VS GENE AMONG SPECIES MAY NOT BE EXTRAPOLATABLE

SYSTEM
- S. cerevisiae
- yeast suppressor tRNA sup4-o
  - 89 bp
    - ∴ simplify analysis
    - most bases important
- put on plasmids
  - YCpMP2 = } inverted gene
  - YCpJA1 = }

PREFERENTIAL REPAIR

WHAT ABOUT DAMAGE SPECTRA?

- bias for TT changes on transcribed strand
- bias removed by rad1 deletion

IF RNAPOL III causing bias then inversion should not affect bias

- inversion - showed NO BIAS

pol δ mutants in 3'-5' exo

	Rate $\times 10^{-7}$
WT YCpMP2	3.1
pol δ- "	213
WT YCpJA1	2.7
pol δ- "	40

- wouldn't expect these to be much different unless pol δ only works on one 5' strand

SPECIFICTY DIFFERS

MUTATIONAL SPECTRA

- AP site
  - Mammalian cells ---
    - AP endonuclease
  -
- Select spontaneous mutations in
  - AP- apn1
  - wt

  } higher rate in apn1
  - most increase in change to GC
  - most due to Apurinic sites
  - says no selection bias bec. of other studies
  - reversion of six lacZ mutants
    - largest increase in AP- strains for AT→TA

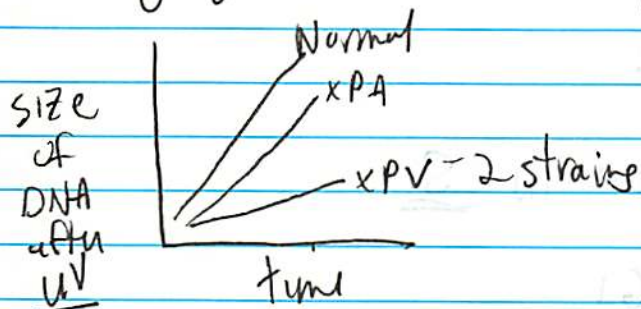
∴ yeast and E. coli appear different

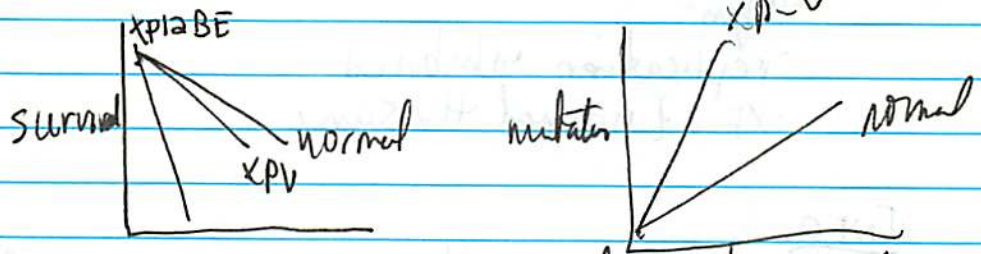
Veronica Maher: Insights into Mechanisms of Mutational Processes by Spectra

XPIIBE XP Variant = XP4BE

Finite life span cells

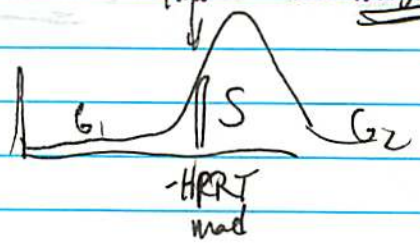
XP4BE
- virtually normal total nucleotide excision repair
- abnormally slow in replicating DNA containing UV-induced photoproducts
- highly sensitive to mutagenicity of UV





- this is when most mutations occur

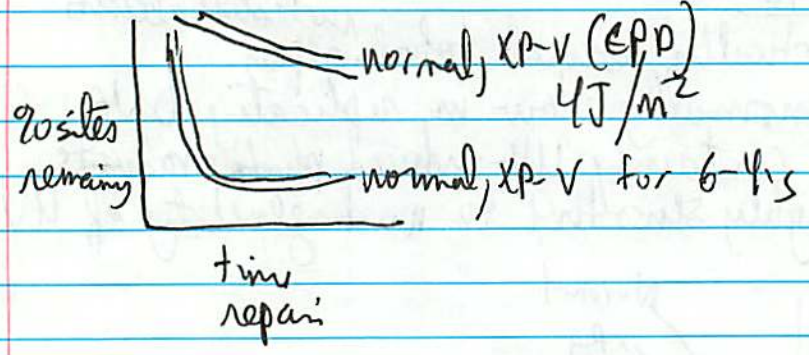
System
- synchronize
- release
-



- in XP-V & normal if irradiate early don't get many mutants, ∴ not error prone repair.

HYPOTHESIS II - DON'T REPAIR IN S

① USED MITCHELL'S ANTIBODIES
② SYNCHRONIZE
TREAT AT S
INSTANT HARVEST VS TIME



-∴ DO REPAIR IN S

-ALSO DID IN G1
-4J/m²
-replication inhibited
-XP-V & normal the same

INFO
-# of lesions are the same in normal & XP-V

SYSTEM
① SYNC
② UV @ 5, 6 hrs before (24 independent cultures)
③ HPRT
- mRNA → cDNA →

~~DISTRIBUTION OF PYRIMIDINES~~

- most of T mutations (TT or CT)
  - Time in CPD's

- ½ of C mutations are CPDs; ½ are 6-4's

- DISTRIBUTION OF PYRIMIDINES

NO REPAIR

	TS	NTS
TT?	18	14
TC?	12	10
CC?	24	12
CT?	6	3

C's - mutations most in ts (~11:1)

T's - 3:1 NTS:TS

XP-V

REPAIR
- most C's in ts
- most T's in nts

NORMAL CELLS
- no repair --- many C's in ts
- repair — C's — in both

Xp-V

NO REPAIR
- way more mutants
- C's in both Ts & NTs
  - C→T's in NTs
  - C→A's in TS — NONE IN NORMAL CELLS

T's
T→C in NTS } v. common ∴ NO A RULE
T→G in NTS

REPAIR - same spectra

	TS		NTS	
	NORMAL	XP-V	NORMAL	XP-V
NO REPAIR				
C→T	48%	3.3%	16%	20%
C→A		30%		
C→G	8%			3.3%
T→C	12%	3.3%	16%	16%
T→A		3.3%		6.6%
T→G				13.2%

Ken Tindall - Discussion

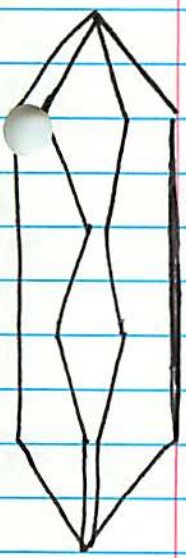
ENDOGENOUS

Free radicals → depurination → polym errors

EXOGENOUS

environ → life style?

repair → replication → mutation



MUTANTS IN SPECTRA MUST BE DERIVED FROM INDEPENT EVENTS

SPECTRA

1. primary sequence
2. selection conditions
3. cell metabolism
   - a. bio-transformation
   - b. DNA-repair capacity
   - c. gene expression
   - d. replication
     - leading / lagging
4. Thermodynamics / kinetics
   - agent
   - DNA sequence

STATS

- JMB 194:391
- Poigorsch - comp out
- Thilly - pending
- Glickman -

Polymorphisms

- NAT
- GST - glutathione transferase

Comments

- worry that spectra from 1 gene is so small relative to total genome

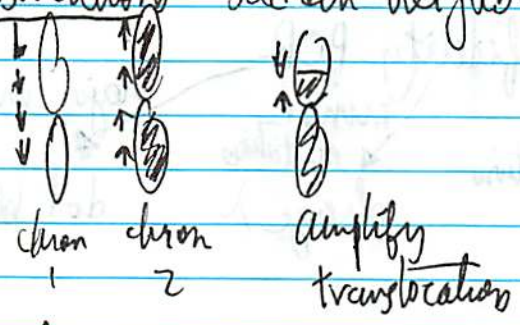
- VDJ recombinases

Tom Cebula: New Strategy for Rapid Detection of Mutations

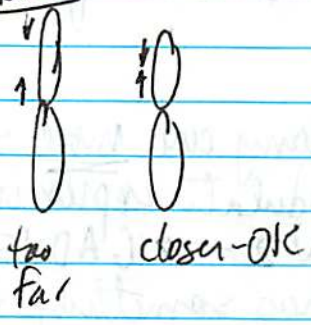
Genotypic Selection
① no phenotypic lag - no wait for expression
② dead cells OK

Norm Arnheim approaches ARG 1992 ABRC 1992
① Single cell techniques - ↓
- worry about PCR artifacts
- PEP PCR
- uses degenerate 15 mers to amplify
- get 30x amplification of 80% of genome

- translocations - sudden neighbor analysis



- deletions



Peter Cerutti - RFLP-PCR & p53 & ras

WHY? { Detection prior to clonal expansion
No phenotypic selection
Detection of low-frequency events

Cells
↓
DNA
↓
preselect fragment of interest
↓
digest to remove wt
↓
high fidelity PCR

add mutant standard ↕

- major mutations → sequence
- minor mutations → phage λ
- major mutations → dot blots/slot blots

- mutant standard allow quantification of PCR

- p53
  - mutants - many are more stable
  - these modulate expression of other genes
    - e.g. MDM2 → GADD45
    - which has something to do w/ growth arrest
  - non-random distribution of mutations in many cancers

Colorectal cancers

- codon 248 is a hotspot - is also highly methylated
- may be due to ENU

Exp

2mM ENU → freq. of mutation at this site is 2.5×10^{-4}

Aflotoxin

- epidemiological factor in hepatocellular carcinoma
- especially if HepB also

- codon 249 preferentially mutated in hepatocellular
- especially common in Aflotoxin areas

ARE THESE MUTATIONS INDICATIONS THAT REPAIR IS BAD?

- in cell culture
  - highest frequency of mutation are G→T at 249 codon
  - also other codons

∴ ALTERED F(x) & HOTSPOT ARE IMPORTANT

Mutations Prior to Clonal Expansion

what about convergent.

Hepatocellular cancers

Bladder Cancers

- H Ras - codons 12, 13, 61
- codon 11 & 12 both have higher mutation frequencies

Wilhelm Bohr

Gene Domains may be correlated w/ loops

no pref. repair in Drosophila

-MFD
-Says ERCC6 is preferential repair gene

ERCC3

ERCC6

Strand specificity

Transcription Independent Gene Specific Repair
① 5' c-myc in mouse DBA B-cells
② ISCL in cisplatin resistant human ovarian cancer

Gene Repair
Tx
Pol
Nuclear matrix
Chromatin structure
F(x) importance in stress

- Mt DNA turnover/replacement

Mt genome
① 1000 copies/cell
② O_2
③ Aging?
④ "No genes for damage processing"

Entire genome
- UV – more or little
- MMS – OK repair
- nitrogen mustard – none/little
- 4NQO – OK
- Cisplatin – intrastrand – no
  - ICL – yes } Syngeot recombination
- O_2 damage -

Strand Specificity of Mutations
- repair
- replication
- sequence context / preferential formation

~~Cell Cycle~~

Assays

① Mismatch Amplification Mutation Assay
Rita

② HUMA assay
- remove microprecipitated DNA
- cut w/ restriction enzyme
- get v. v. little non-digestion

③ DGGE - can sep. frameshifts too
④ SSCP
⑤ Constant denaturing capillary electrophesis

Ray Tennant

Genome Wide Insertional Mutagenesis

1. insert exogenous DNA fragment →
2. study phenotype
3. clone out region of insertion

Terocenase cDNA

- use as exogenous DNA
- this corrects albino phenotype
- can use to study promoters

Mutations induced

1. insertion
2. deletions in insertion site
3. structural rearrangements like translocation

- suggests that this may pick up insertion hotspots

Examples

- homeotic mutations
- abnormal kidneys
  - gene has TPR repeats as do many cell cycle proteins

Agouti -

- many many alleles or many linked genes?

W.T. — hair: black yellow black

- overexpress = yellow
- KO = black

- some yellows have phenotype of NIDDM

Agouti
- 131 aa protein
- signal peptide
- 4 exons
- highly basic

Alleles
a = insertion in ivs w/ mouse DNA & retro
a^t = mouse DNA hops out
a^y = 150 Kb deletion upstream of agouti
= Raly gene
= mutant may make agouti from this genes prom.

John Sdrunenti:

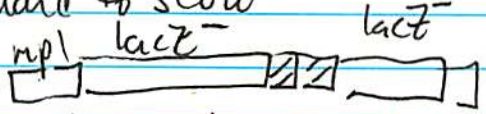
Interested in gene conversion in mammals

Suggests much of evolution is punctuated equilibrium

What happens after gene duplication?
- Gene conversion
  - can occur betw. any two related seq. in genome
  - used in
    - mating type loci conversion in yeast
    - Ig diversity in chicken
    - Trypanosome antigenic variation

- In Mammals
  - more homology among than betw. duplicates
  - may be responsible for many diseases

- Frequencies in Germ line
  - hard to score

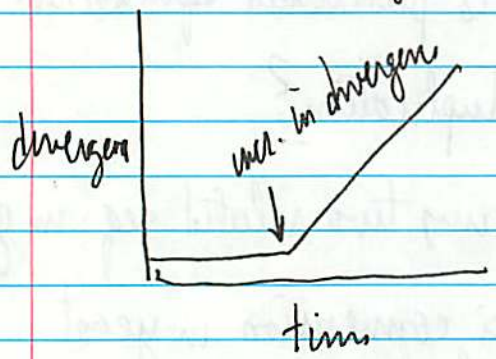


  - need transfer to get lacZ⁺

~ 1% conversion

Mechanism to Inhibit Gene Conversion?

More similar genes have higher conversion



what is frequency sectors of mitotic events?
↓ not as high as background

WHAT ABOUT BACKGROUND?

Mutagens

Chlorambucil ... 73% vs 2% non-tx
what about background.

② ENU - >5 fold increase

③ EMS → 2-3 fold (even 60 days later)

Heinrich Malling

- Alive vs dead
- * - Mutation Frequencies in hemi vs. homozygotes
- Mutations in tandem arrays may be removed or increased by 'conversion'

BIASED GENE CONVERSION

Human polymorphisms important for mutation frequency
- GST -
  - 5% of pop. has <20% average activity

Flow

Intraindividual variation

Aknoll et al

lacI transgenic mice

↓

grow

↓

isolate tissues

↓

DNA

↓

λ

↓

Ecoli

↓

lacI mutants

- different spectra in liver & spleen

Adler E Fijalkowska RM Schaaper

dnaE α - polym
dnaQ ε - proof.

Genetics 134:1023, 1039, 1031

Q919 - transition bias
E919 transversion bias

Dick Albertini : Human Genetic Disorders & Mutation

Steve Sommer – Factor IX ... Hemophilia B

① common, X linked recessive
② small reduction in activity produces disease
③ diagnosis easy
④ lethal so most mutations recent
⑤ small (~2x below low range of normal) decrease leads to disease
⑥ fetal development unaffected
⑦ 95% of families w/ severe/mod. disease have indep. mutations
⑧ no heterozygote advantage
⑨ most of domains unique

except in mild disease

METHODS

PCR – GAWTS

RAWIS/GAWTS – PCR w/ T7 prom. in primer
- make tx
- sequence SS-RNA
- can use to make in vitro translation

RESULTS

- get candidate mutation in 95% of cases
- 2/3 of mild disease due to descendents of 3 ancestors

How show this?

279 mutations
203 independent (distinct)
9 second change
92% 186 coding
8% 12 splice (x)

Ts at CpG	~30%
Ts not at CpG	~30%
Tv at CpG	4%
Tv not at CpG	20%
Microinsert/delet	8%
Deletions	6%

Estimate mutation rate

$$\frac{HABx}{CxD} = \frac{freq}{target\ size}$$

H = rate of de novo HemoB
A = fraction in coding region
Bx = fraction in coding that are of X type

1st 48 sites
47 at conserved positions
25 at residues conserved in Fac 7, 10, prot C, Fac 9
9 " " " " Fac 9

- suggests 40% of residues can't change
60% can

SAYS PATTERN IS ANCIENT ONE
BECAUSE IF YOU TAKE RANDOM
SEQUENCE GET 40% GC &
UNDERREP. CpG.

- not much age effect

David Yandell RB & p53

Physical Spectra → Biofeltes → Methodological Filter
Selection
Endog
Speeus
Sep
Ongen
locus

Rb 13q1.4
- 928 aa
- 5-10% of all new cases = inherited
- 1/10 who inherit predisp. don't have it
-

New Germinal Mutations
- may be from gametogenesis of parents
- " " " embryogenesis

- 8:1 ratio of paternal allele biased for germinal mutations

p53
Li Fraumeni syndrome

WHAT IS POP. DIVERSITY.

Germinal vs. somatic
- may have different biological targets

	Germinal		Somatic	
	p53	Rb	p53	Rb
MISSENSE	6	8	77	1
NONSENSE	8	79	27	43
PROMOTER		5		

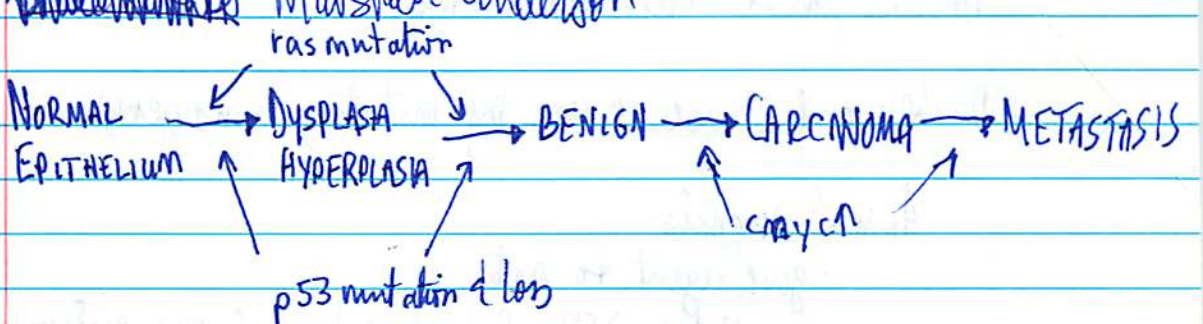
p53 - Biological Target

Low penetrance -

what about missenses that don't cause problems?

non small cell lung tumors

[redacted] Marshall Anderson



GENETIC CHANGES LEADING TO PROTOONCOGENE ACTIVATION

① pt mutation
② insert/delete
③ gene amplification

SAYS BEYOND COMPREH. THAT CHANGE AGENTS COULD SELECTIVE ENVIRON.

Inactivation of 2nd allele.
- sometimes happens by dominant negative
  - because its a dimer
  - and because mutants are usually more stable

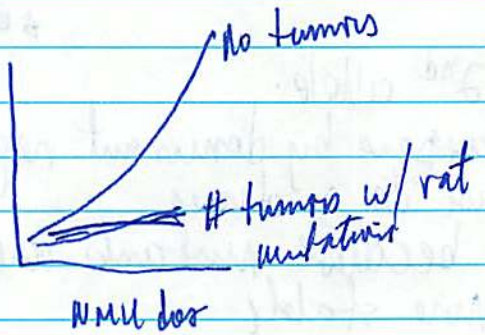
Helmut Zarbl = Activated Ha-Ras-1

Do chemical cause cancer by mutating oncogenes

Animal models-
- gave agent to rats
- NMU - 85% of tumors have C-ras activated
  - 61/61 of these have mutation at codon 12

DMBA - 15% of tumors have C-ras activated
- all at 61st codon

Gould



Other possibilities
NMU selects for pre-existing mutants

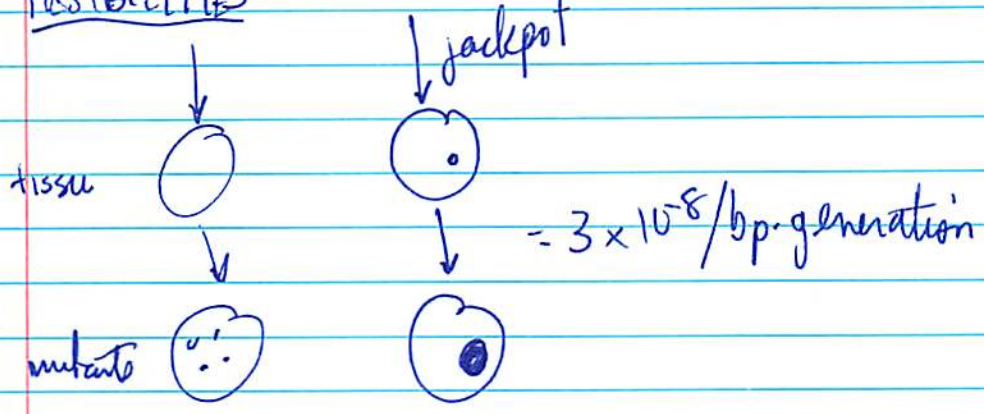
METHOD
① measure induction of mutants after NMU
② MAMA
- allele specific PCR w/ mismatches
  - wt - two mismatches - } one mismatch is much more efficient
  - mut - one " " - }
- only 1st cycle is important

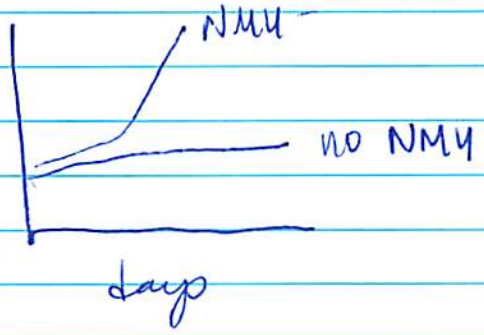
Why not use PCR of opposite strand

RESULTS

- ~300 mutant cells / 3×10^6
- $= 10^{-5}$ mutation rate

POSSIBILITIES





① NMU not inducing significant fraction of mutant cells

② fraction of NMU negative rats doesn't change

③ # of sectors w/ mutants doesn't increase

GGGAAA
CCCTTT

Roger Wiseman p53 tumors in mice

p53
·393 aa
·

~~Transgenic~~

Cultures
mice = wt
= hemyzygous } not many problems
= null - many chromosomal segregation problems

Transgenic mice - w/ p53 mutation
- wide spectrum of tumors
~~mammary adenocarcinoma~~

WHY ONLY INBRED STRAINS?

Do chemicals initiate carcinogenesis?
Animal models
- limit genetic variability
- reproducible induction of specific tumors

Cross two strains
- can use polymorphism to track mutations
- use microsatellites to map loss of alleles
- PCR p53 - multiplex
- SSCP - sequencing gel

run each G next to others

Early Onset Breast Cancer
- also associated w/ ovarian cancer
- BRCA1 is gene - Chr. 17
- Many Clair-Ling mapped to region
- also be mapped w/ mucos. sporadic tumors to same region

Finding Genes in Region
Direct Selection
- Tissue specific cDNAs
- multiple overlapping cDNAs
- must block vector

Tom Skopek

Butadiene → Diepoxide
Epo Monoepoxide

HPRT locus
- Diepoxide induces many inserts/deletes
- also many frameshifts induces

Ethylene Oxide similar

Stable allele
↓
Unstable allele
↓
"Pre" mutation $\xrightarrow{\text{92\% up / 8\% down}}$ mutation → FMR 5' methylated

no FMR protein
↑
tx suppressed
↑

FMR-1
- RNA binding domain
- assoc. w/ cellular matrix

MYOTONIC DYSTROPHY MT-PK
- also has anticipation
- autosomal dominant
- severe congenital form in affected children of unaffected? mothers
- mapped to chromo 19
- scanned all CG containing tuplets
  - GCT?
- cloned & sequenced
- in 3' UTS

Normal	5-23
Congenital	500-5000
Juvenile	226-1000
Adult	50-1000
Subclinical	

AMPLIFICATION
- substantial from ♂ - offs. ; higher from ♀ → off
- around 80 copies = unstable
- somatically unstable

Tom Caskey

Amplification of Triplet Repeats

- Found tetrameric repeat in HPRT
- Also found polymorphic in Androgen receptor

SBMA 40-50 repeats unstable in Spinal Bulbar Muscular Atrophy
~~Androgen receptor~~

Fragile X recessive
80% males - some retardation
30% female " "
some "normal" males transmitted trait
- CGG repeat - in 5' untranslated
- 20-40 repeats in normal population
- 50-200 " " in relatives of affected individuals
- 720b " " " affected males

- in mothers of affected males - more repeats = more likely a offspring will have it

WHAT ABOUT OTHER TRIPLETS?
- 8-9% of changes are decrease
- male to offspring transmission rarely have significant amplification

- repeats > 200 associated with abnormal methylation
- risk increase as go down pedigree (Amplification)

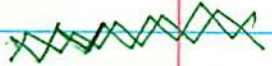
steady state RNA lower when more repeats

HUNTINGTONS CHOREA

- 10-50 copies
- disease = 50-100

FRAGILE X - 2 = CGG

Collecting more



What about RIPPING?

Ken Turtletaub Adduct Dosimetry

Adduct detection as biomarkers
- High doses - OK signal
- Low doses -

AMS - Accelerated Mass Spectroscopy
- measures amt. of different isotopes directly
- only use C.

- 3H ok

Heterocyclic amines & benzenes

Dioxin

Adduct removal kinetics
-

Combine w/ HPLC
Combine w/ electrophoresis & quantify adducts in specific bands

Bill Lee

BUT WHAT ABOUT UNIVERSAL EXPOSURE TO SAME MUTAGEN

Down syndrom
- suggest endogenous affects are most important

Germ line risk
① gametes may be selected
② homozygous recessives "lost" if q is low
③ interspecies comparisons not necessarily effective because of diff. in metabolizing enzymes like p450.
④ intra species risk must look at germ cells because of w/in diff. in enzymes

Mutations in heterozygotes Adh
- says G x E is important
- also says dominance depends on G

Harvey Mohrenweiser

Gene Mutations
- new alleles in offspring

Factor VIII Hemophelia A 67% to substitution
Factor IX " - B 75-80% " "
DMD Dystrophin
steroid sulfatase

CGN
NCG
GNN
NNC

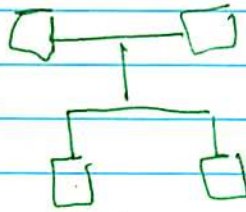
- direct repeats
- cleavage sites
- repeat elements

↓
N

J. Hall

Mosaicism

A/B/x^ C/D



X*/B X*/C

- mutation occurs during development
- 10% of cases for many diseases appear to be mosaicism.
- its the mosaics parents who are important to study

Sid Aaron

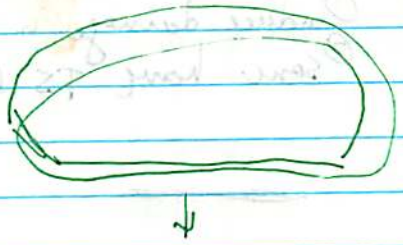
Drosophila
- sperm

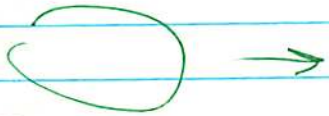
CpG frequency coding vs junk

- α-globin -  CpG : GpC = 1:1

PCR amplify newly synthesized DNA?

- kill methylation
- density label
  - C^{13}
  - N^{15}





DNA turnover

① grow cells
② add C^{13} thy - N^{15}
③ separate replicated / semi / non
④ quantify C^{13} in bands of DNA

Is there anything on differences in phenotypic effects (such as leakiness) of insertions vs. deletions?

- would one expect cpt mutations to be more damaging than others - do cpts play a f(x)al role?

- Is the spacing betw. direct repeats f(x)ally significant.

= ung vs sperm?

- selection detected by examining mutation spectra
- what about selection during presence of damage
  ① induce damage in population
  ② some have TS lesions some NTS

Selection
  ① examine mutation sites
      look for aa bias

- ~~Take mutational spectra~~

- ~~p53 in yeast?~~

- ~~in cancer mutations~~
  ~~- can't exactly look at mutations to say something about Rx because mutations may be consequence & not cause of phenotype~~

~~use Ab to pull out Arch rect~~

~~Frequency of tetranucleotides~~
~~E. coli vs Yeast~~
~~- NAR 20: 1663.~~