

Hanawalt Lab Retreat 1995

Phil Hanawalt - K

Graciela Spirak - G Features of TCR

Allen Smith - -expressed genes, txs

Ann Ganesan - -only pol. II

Joyce Hunt - -regs. elongation

Brian Donohue - -specific for lesions that block tx.

Masahiko Sato - -requires TRCFs

Jim Ford

	<u>Bacteria</u>	<u>Eukaryotes</u>
Fred Christians	mfd	CSB
Krista Bowman	mutS	CSA
Yafe Lin	mutL	MSH?
David Koehler		MLH?

Fred Christians

Krista Bowman

Yafe Lin

David Koehler

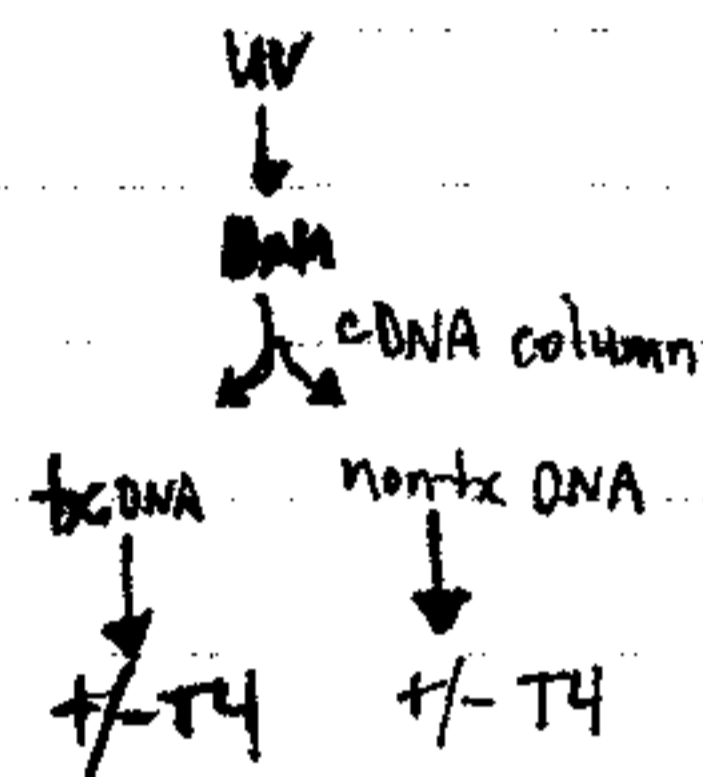
Jonathan Eisen

David Gowley

Justin Courcelle

Larry Loeb

Use cDNA to pull down fragments of expressed strands?



Jim Ford - p53

p53

- induced w/ DNA damage
- transcription activator (p21, mdm2, bax, GADD43)
- interacts w/ other proteins (mdm2, helicases, TBF)
- induces apoptosis

Role in NER

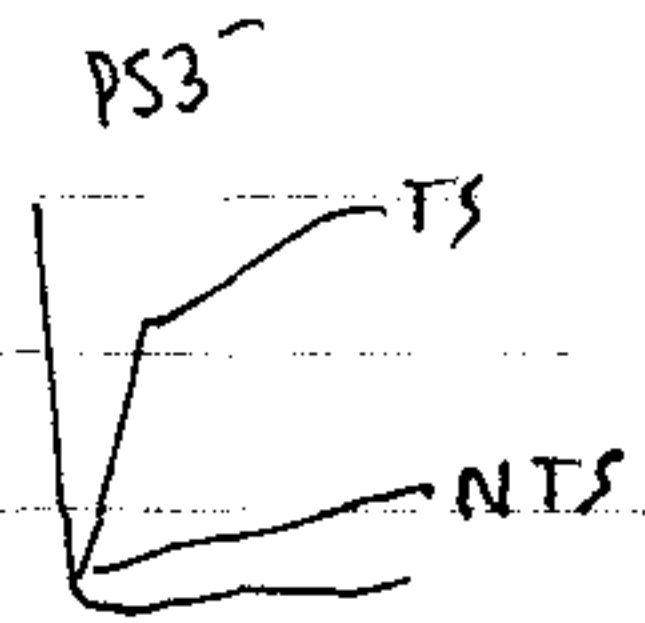
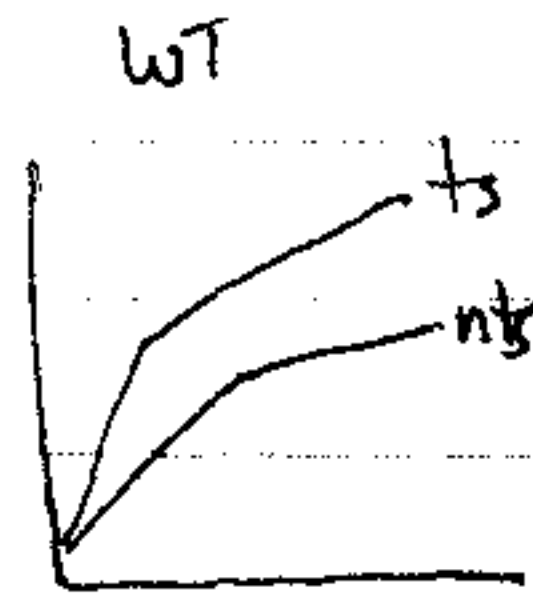
- when p53 inhibited by HPV → decr. NER
- inhibition of GADD45 (downstream of p53) → UV sensitivity + DNA repair defects

Induction

<u>Bacteria</u>	<u>Euks</u>
RecA/SOS	p53

P53 mutants

- defective in repair of non-txs
- defective in UV induced apoptosis



↑

How might P53 affect repair?

- ① tx. regulation
- ② interact w/ repair prots.
- ③ binds damage?

What induces p53?

REMEMBER ... AFFECTS

SHOULD REMEMBER

THE WHOLE

ORGANISM NOT

JUST CELLS.

YAFE LIU

Bcl2

- 239aa

- 26 KD

- membrane protein - mito, ER, nuclear, cell membrane

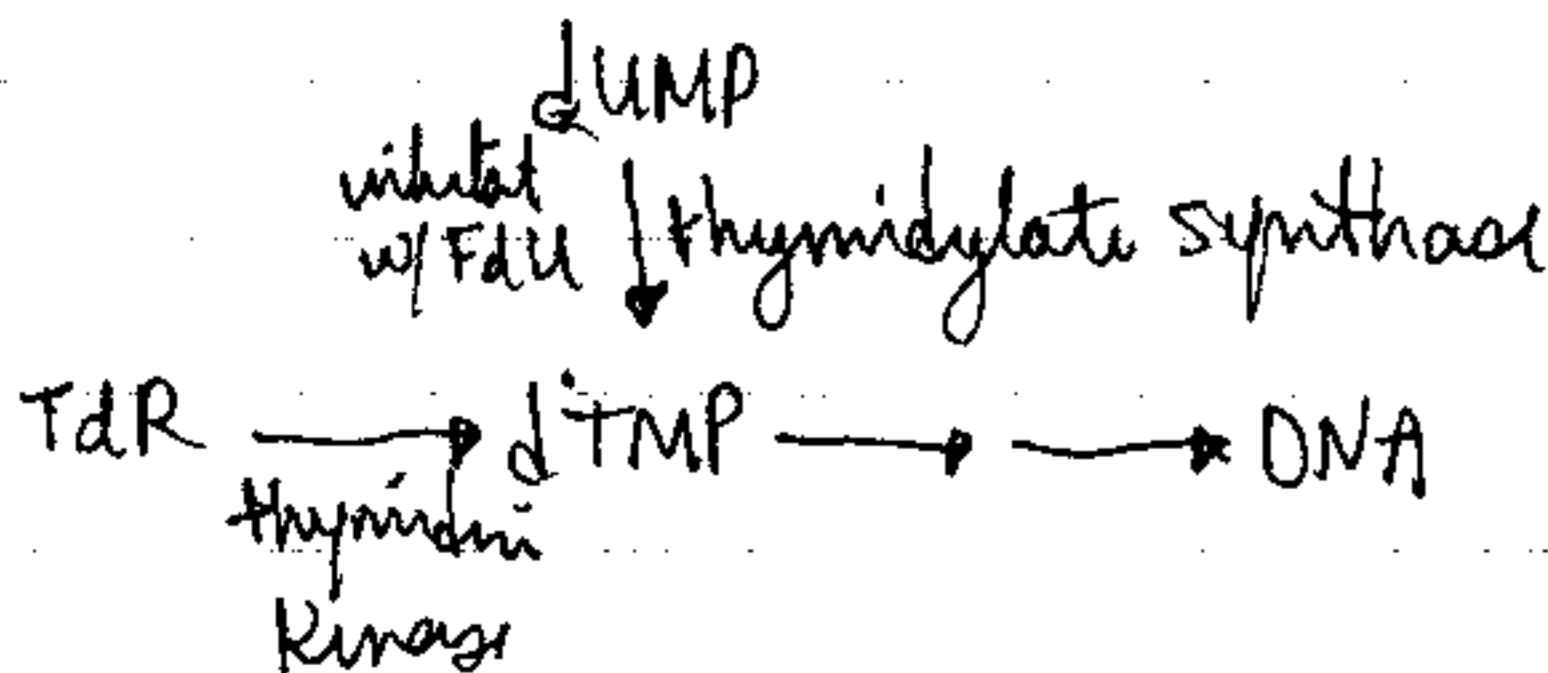
Larry Loeb

RANDOM MUTAGENESIS

Genetic Selection from Random Sequences

1. Tetracycline promoter
2. B-lactamase
3. Herpes thymidine kinase
4. Reverse transcriptase
5. DNA pol. B
6. Tag DNA polymerase

Thymidine Kinase



- Herpes enzyme works slightly diff. from human
- So...select even more

Heat stable mutant

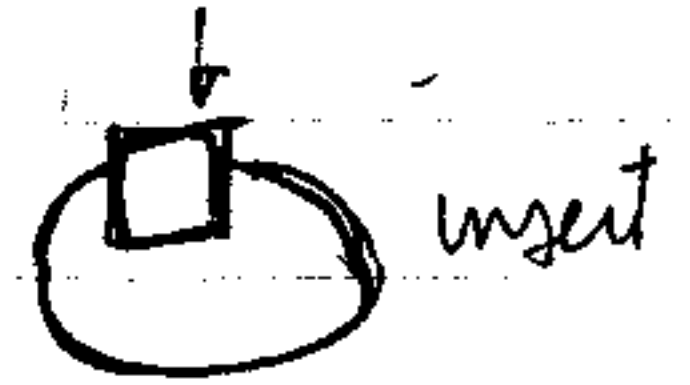
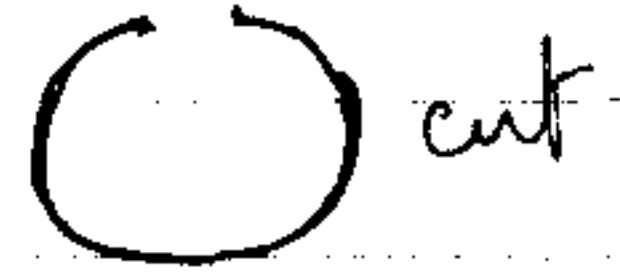
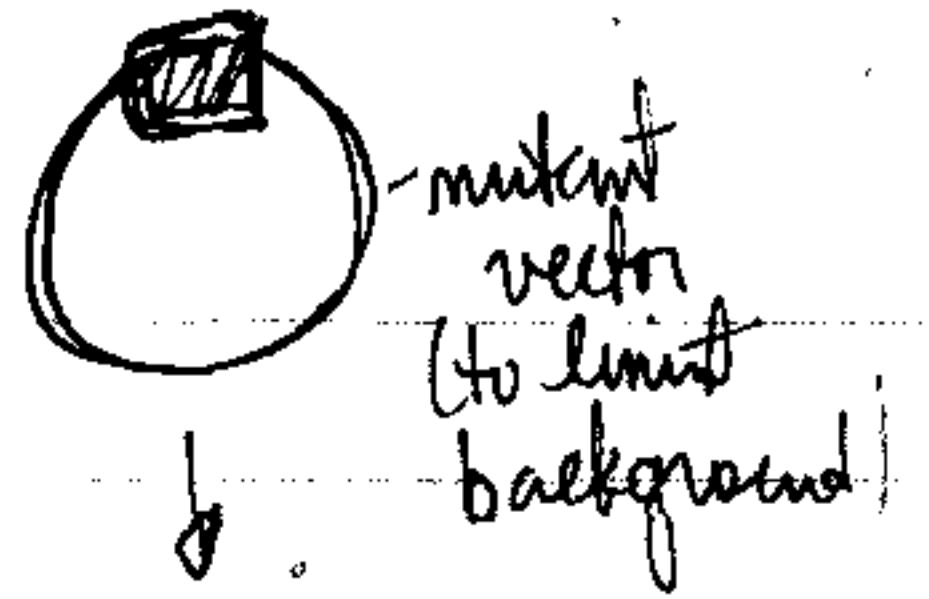
- 3 amino-acid changes from w.t.
- apparently no single or double Δ 's lead to heat stability

polA^{ts} ... can be complemented by polB, or HIV RT

- are there sites that cannot be mutated
- can use to screen for resistance to drugs

Tag

- can use same complementation assay



Easier to increase activity than stability

FRED C.

Alkylation of DNA

- MNNG & MNU etc

- many diff. lesions (alkyl groups, AP sites)

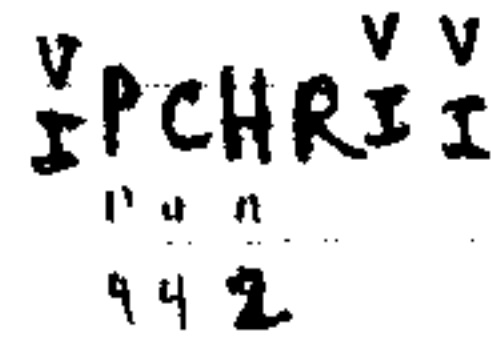
- many repair pathways

Alkyltransferase

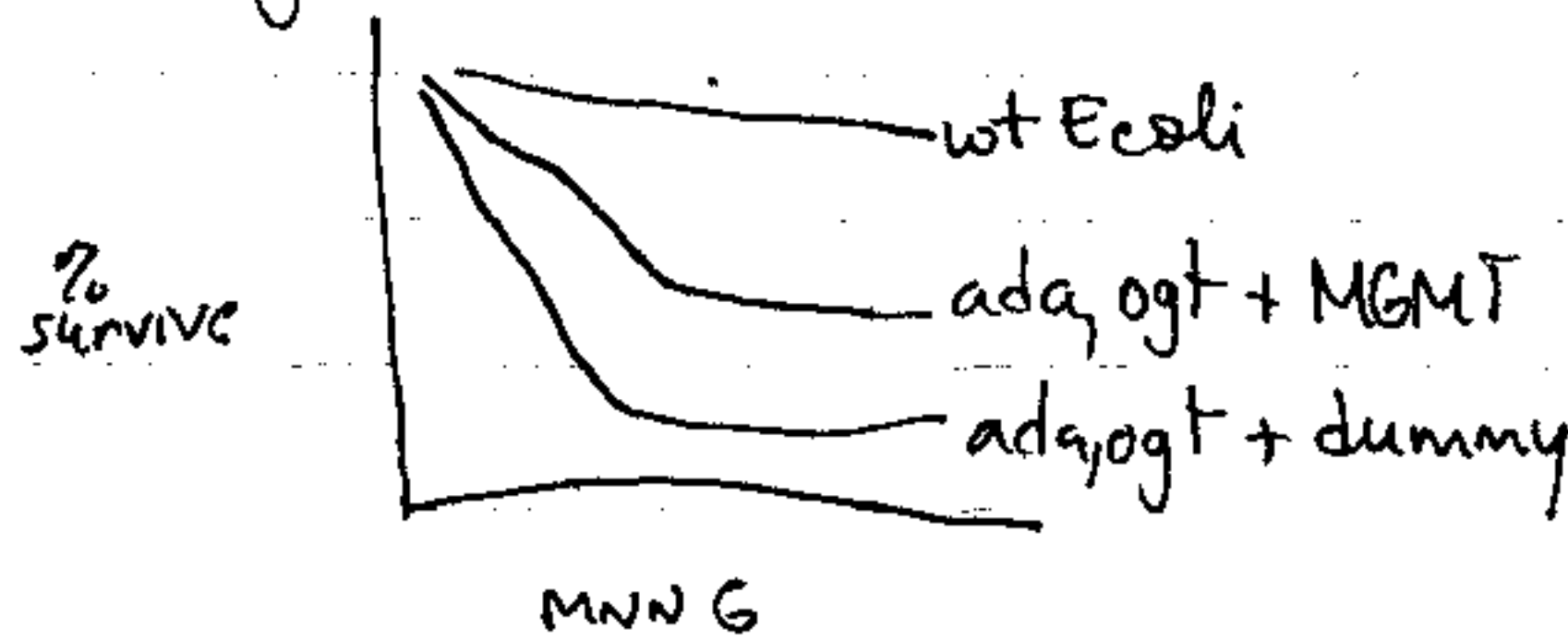
- acta crystal structure solved

- but C (active site) buried

- near phylog. conserved site

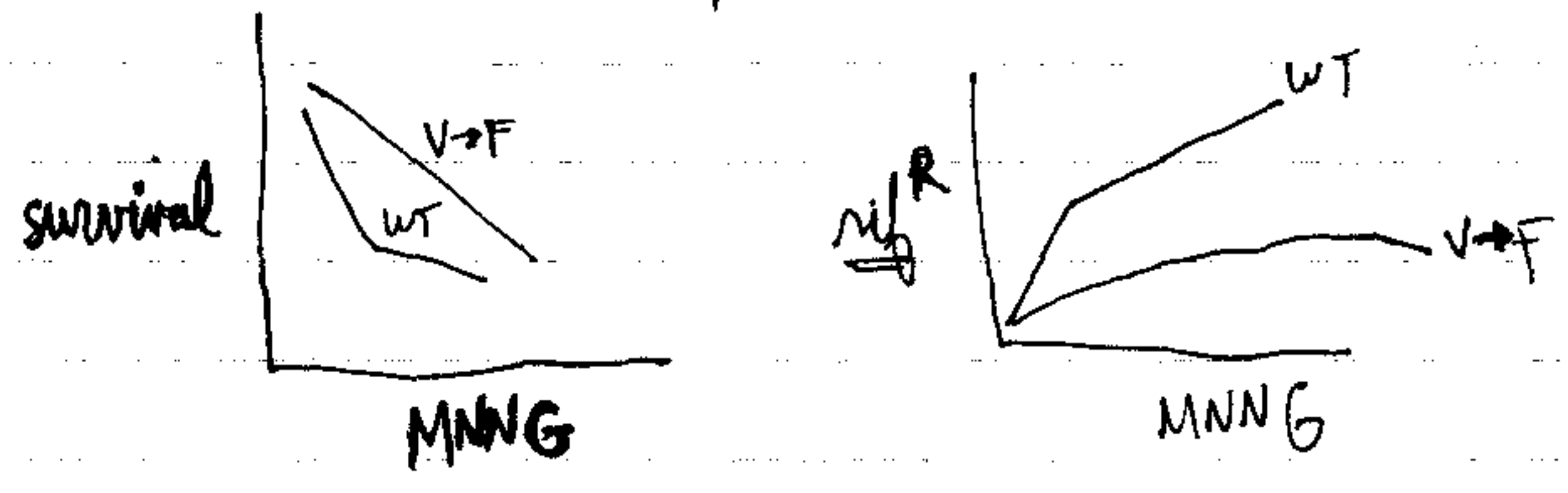


MGMT library construction



But using multi-dose -- get bigger difference between wt & dummy

One of the changes is over-represented
 $V \rightarrow F$ at one position



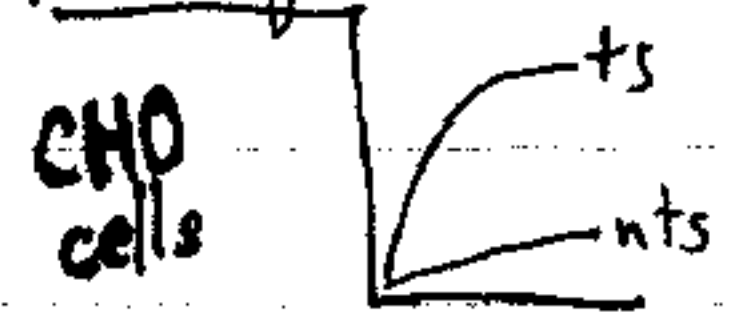
Allen Smith

Lessons

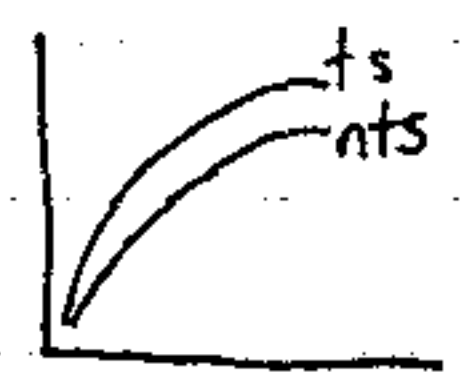
- 6-4s
- CPDs
- other - can avoid the multi-lesion problem
- psoralens - can turn on and off

TCR

- how define



some human genes



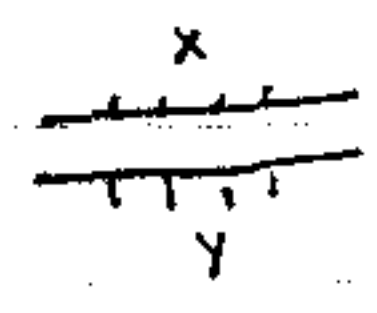
} sometimes strands very similar

- is there a problem w/ doing % repair vs. # lesions repaired

$x = \# ts$
 $y = \# nts$

$\frac{x - t \Delta x}{x} = \% \text{ rep.}$

$\frac{y - t \Delta y}{y} = \% \text{ rep.}$



Krista Bowman

- recognition machinery in TCR
- diff. lesions --- is diff. in repair due to diff. tx affects

BPDE

- laser assay not very reliable

XPC

- relatively UV resistant (vs. other XPs)
- repairs TS only
- similar repair phenotype to RAD16 & RAD7
- homologous to Drosophila protein
- v. hydrophobic
- purifies w/ HWRAD23

RAD4?

load diff. amounts
of DNA to see if
get same result

What is repair
phenotype of RAD4?

Specific lesion assay
