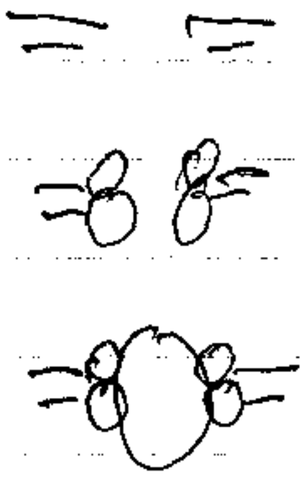


SCID defect
- is only in chromosomal repair

Ionizing radiation response

XRcc1	ligase enhancing
XRcc2	
XRcc3	RAD51

VDJ recomb. and DSBR deficient

~~XRCC4~~	
XRcc5	Ku80
XRcc6	Ku70
XRcc7	p350

SCID
- DSBR defective but not gone
- extrachrom. recomb OK

Homologous vs. Non-Hom. Recombination

Hom. Recomb
- meiotic recomb.
- DSBR in G2

Hom. Recomb - can also work by single-strand annealing of tandem repeats

Rid Sonny

—ATCGT C—
—T AGTTG—

↓

anneal

—ATCGTC
—TAGTTG

↓

ligation of left end

↓

excision of GT

↓

fill in

↓

ligation

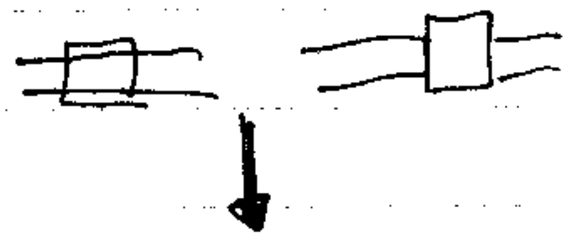
ATCGTC
TAGTTG

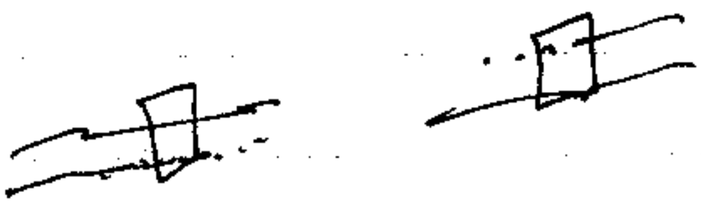
---

break

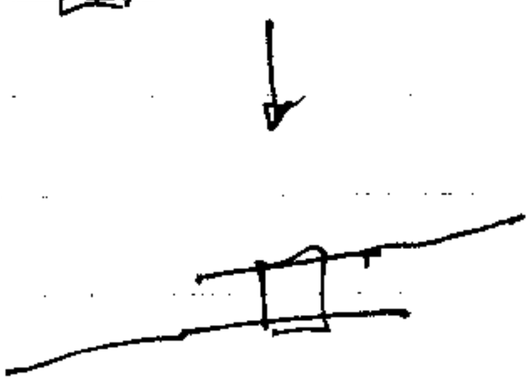
↓

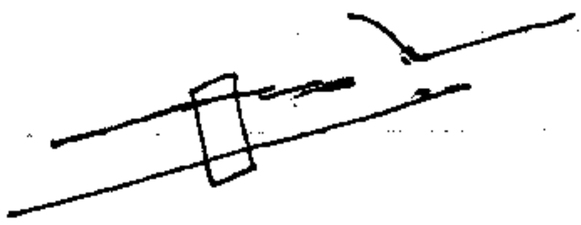
exonuclease





annealing of conserved regions



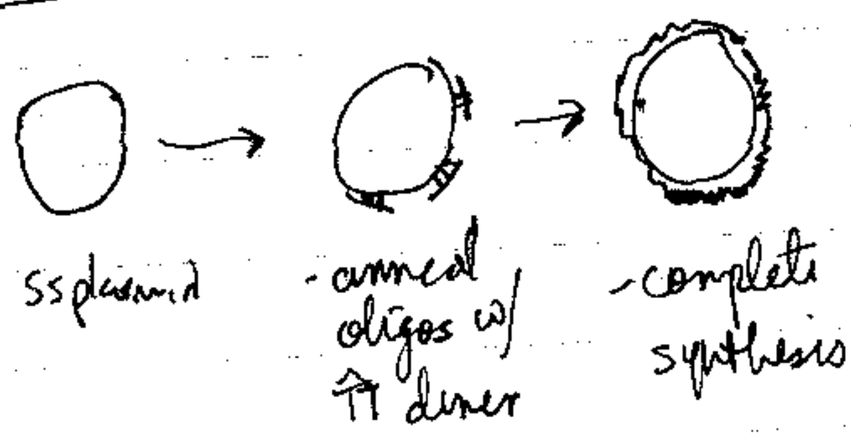


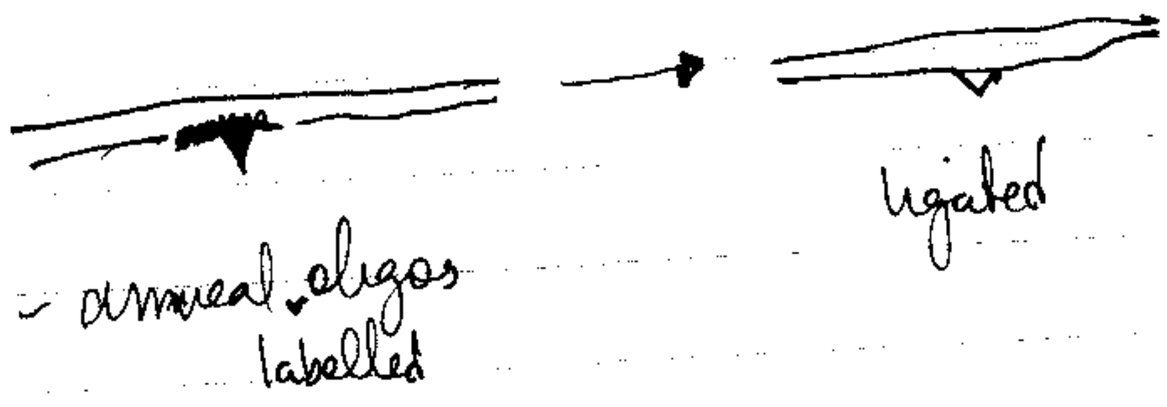
Chris Parias - Synthetic Substrates for In Vitro Assays

NER Reaction Products
- incorporation
- excision

Repair Synthesis Assay - R Wood

Excision Assay - Developed by A. Sancar





- resolve excision products on sequencing gel

Other synthetic analogs
- universal phosphoramidite for attaching any amine reac group

- BIOTIN, Texas red, Rhodamine, Fluorescein, Dinotrophenol

- why not use

- make labelled oligo
- anneal
- synthesize
- treat w/ agent
-

Steve Tronick - Expression of Recombinant Proteins

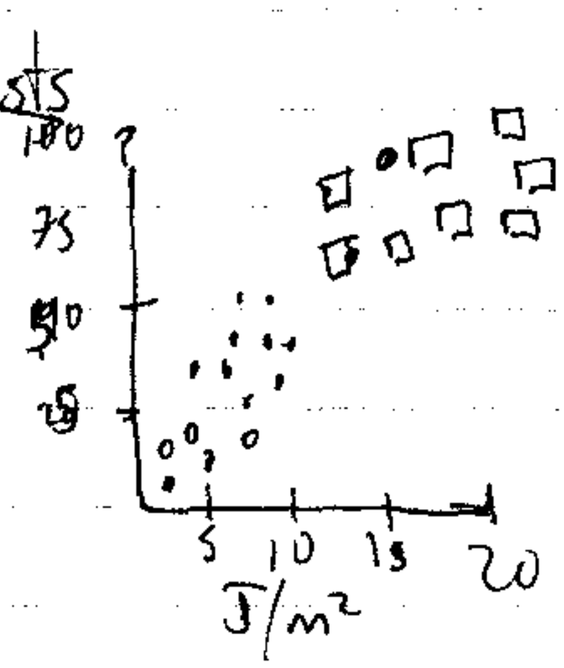
sounds like RAD5

J. Cleaver - XPVariant
- no detectible NER deficiency
- but no diff. from other XPs in phenotype

- also highly mutable by UV

- explanation
  ① over-exposure to UV?

Fibroblasts



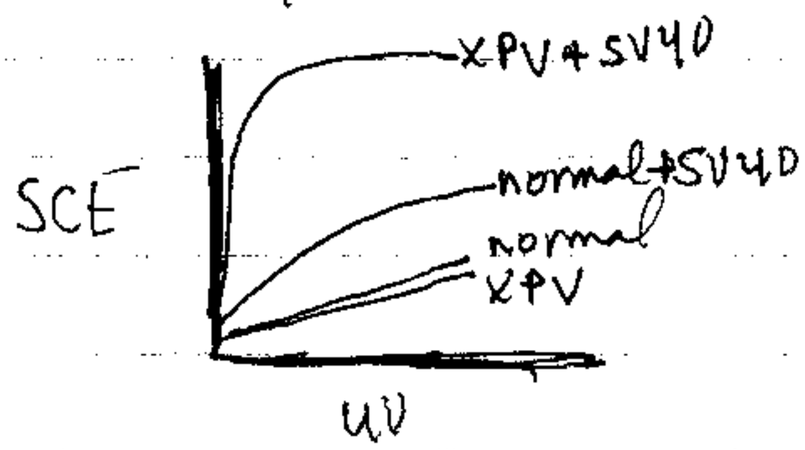
- at LD50
- = xp
- = normal

Post-Replication Repair

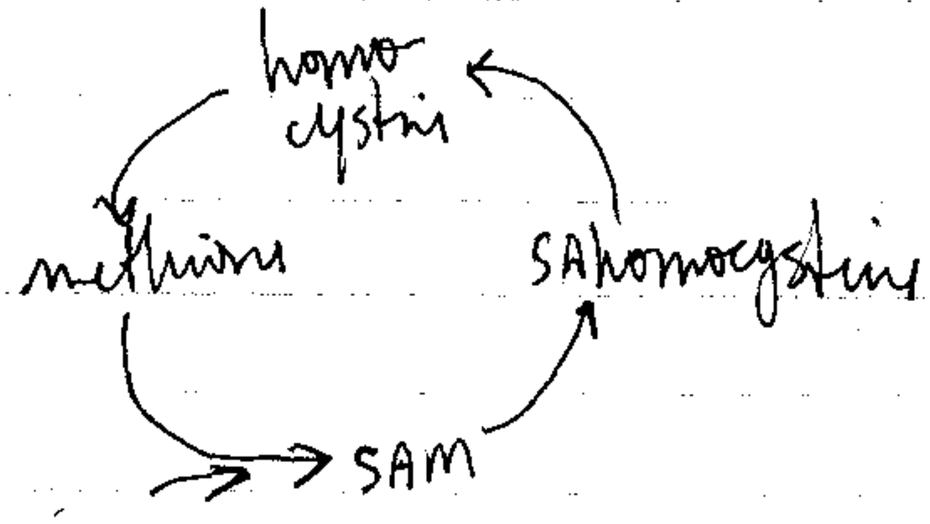
- gap ≅ 1000 bases
  - similar to mismatch repair

XPV

- average mol. wt of new DNA is smaller and smaller w/ incr. UV



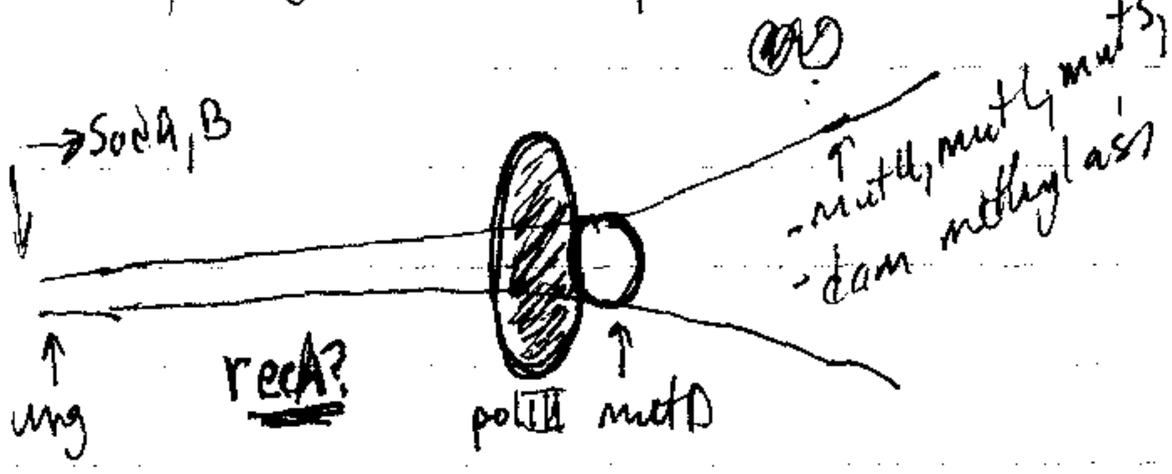
- Caffeine incr. UV sensit. of XPV in S phase
- this is even more so in SV40 transformed
- complement this w/ cDNA
- sequence
  - Irb in Drosophila = p200 = similar to gene in methylation pathway



Jeff Miller

3 parts - Mutators

mutator = cell w/ higher rate of spont. mutation



human mutators

- try and find other mutators first

① use papillation assay
- grow cells on glucose + lactose
- use lac⁻ cells
- # of papillae = measure of mut. freq.

② wanted to eliminate common mutators
- so use six diff lac⁻ strains

mutA
mutM } oxidation damage repair
mutY
mutC
mutT

: 8-oxo-dG glycosylase
= GA glycosylase

: prevents 8-oxo-dGTP from being incorporated

mutY mutM double mutant = v. large incr. in mutation

Human

mutT = sekiguchi has one
: Miller has another

mutY = cloned homolog
: 16 introns
: 535 aa (41% ID to E.coli)
: chromo!
- screening tumor lines for defects here

mutM = cloned fragment

MutA + MutC

- both incr. AT→TA GC→TA transversion
- both incr. transitions in mutH & mutL background
- mutA mutC double = slightly higher

CASCADE OF ERRORS

: mutator tRNAs
- anticodon of gly changed to reading ASP
- in mutD
ASP → GLY is mutator
- % in small % of mutD's = mutators
- DOMINANT

w/out. T
you decr. mispairing
of tRNAs.

Potential Source of Spontaneous Mutations
- v. low rate in wt cells
- might be due to natural error rate of RNA pol.

Archaea
- Pyrobaculum aerophilum (related to thermoproteus)
- Hyperthermophilic, 100°C
- Facultative aerobe
- 2MB genome
- ESTs

3000 runs = 50% of genes

20% matches to public databases

What

mut Y	XPB, D, G
mut T	XRCC1
mut 42	06
RAD51	
RecF	
RecQ	

BARD

C. Kirchgessner

- SCID mouse VDJ recombination deficient
- hypersensitive to ionizing radiation
- complemented SCID phenotype w/ segment of chromosome 8
- mapped to 1 region near centromere

DNA dependent protein kinase - 3 proteins
Ku p80 - complements XRS 5
Ku p70 - maps to chrom. 22 (∴ not SCIDS)
p350 - catalytic

p350? - maybe SCIDs
- maps to correct region
- defect. in SCIDs cells
- present in all complemented cells

p350:

DNA-PK
- phosphorylates many tx. factors, RPA, p53, & RNA pol II

- p53 phosphorylation thought to stabilize it and allows induction after ionizing radiation

- p53 increase is present in SCIDs
- RPA phosphorylation also increases as expected in SCIDs