Life in the Serendipitous Lane: Excitement and Gratification in Studying DNA Repair

DNA Repair Interest Group

History of DNA Repair

June 20, 2006

Dr. Stuart Linn

University of California, Berkeley

Serendipity

Coined from *The Three Princes of Serindip* (Sri Lanka), a Persian fairy tale in which the princes have an aptitude for making fortunate discoveries accidentally

The formative years

Caltech 1958-1962	Stanford 1962-1966
Linus Pauling	Arthur Kornberg
Richard Feynman	Paul Berg
George Beadle	Phil Hanawalt
Norman Horowitz	Joshua Lederberg
Norman Davidson	Charles Yanofsky
Jerry Vinograd	H. Gobind Khorana
Henry Borsook	**I. R. Lehman**

Postdoctoral and beyond

Geneva (Cambridge) 1966-68
Eduard Kellenberger
Richard Epstein
Werner Arber
(Sydney Brenner)
(John Smith)

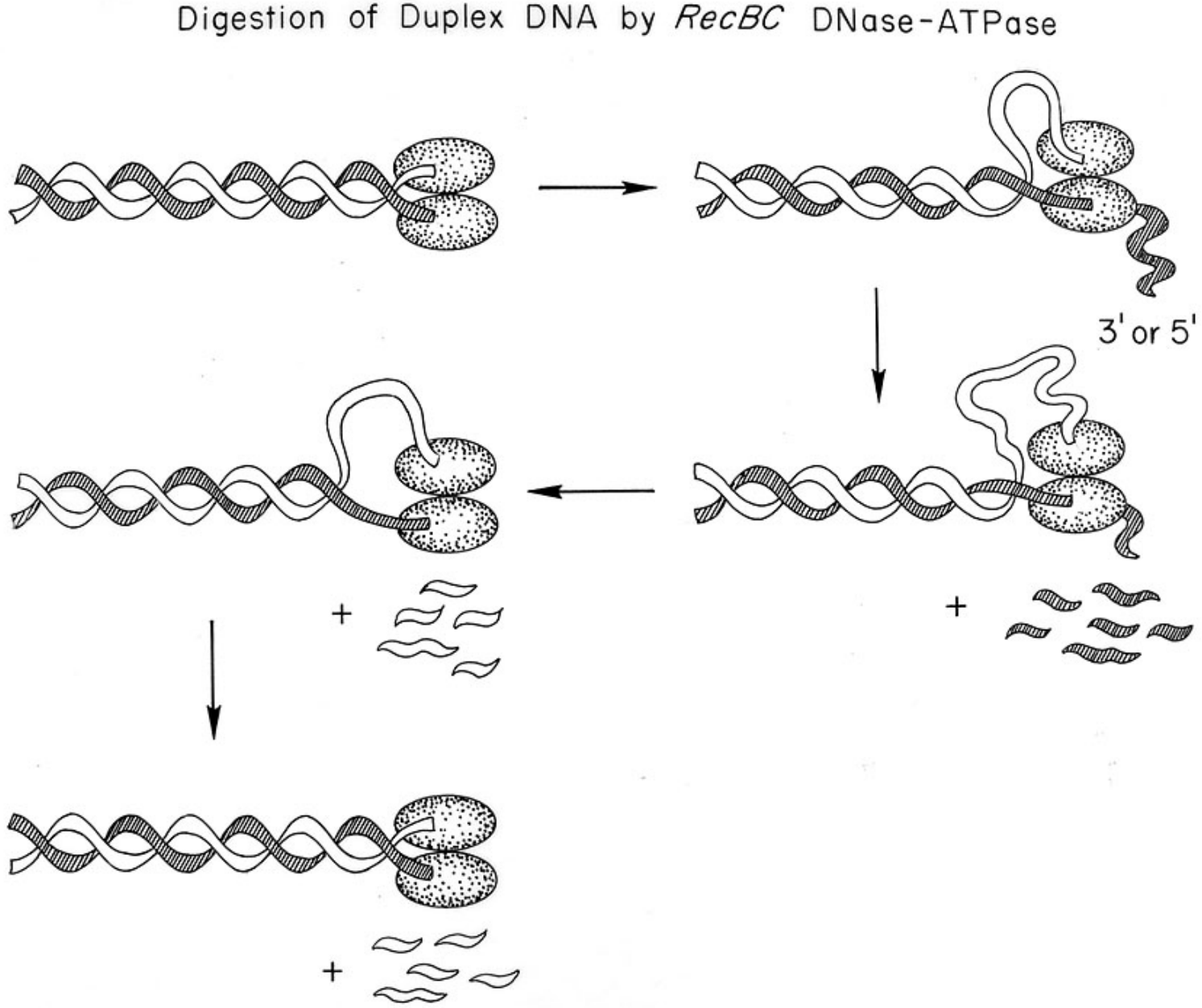
London 1974-75
Robin Holliday

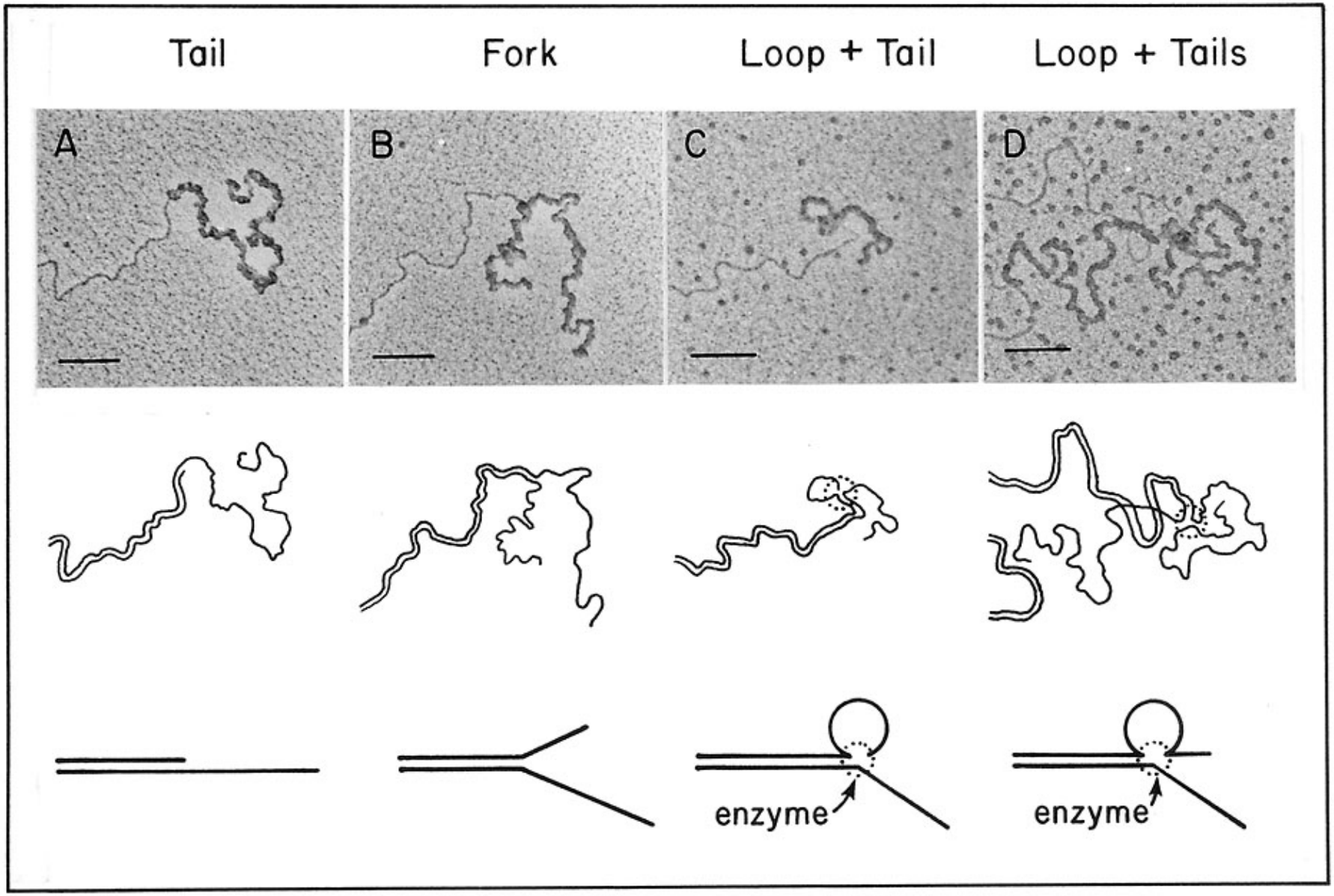
Oslo 1982
Erling Seeberg

Berkeley 1968-
Harrison (Hatch) Echols
Bruce Ames
A. John Clark
Edward Penhoet
Robert Mortimer
Symore Fogel

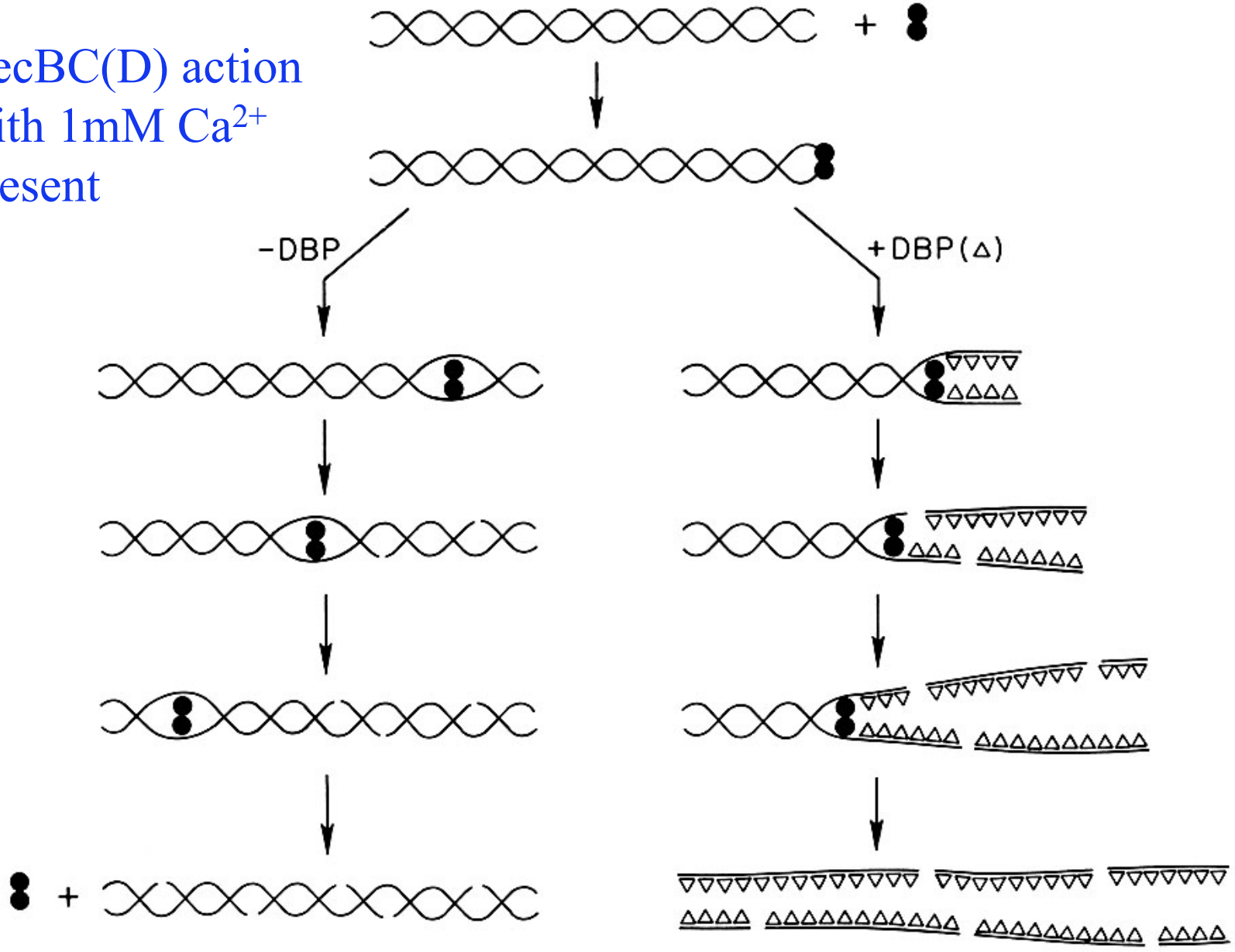
Aviemore, June 1973
Matthew Meselson
Charles Radding
Bruce Alberts

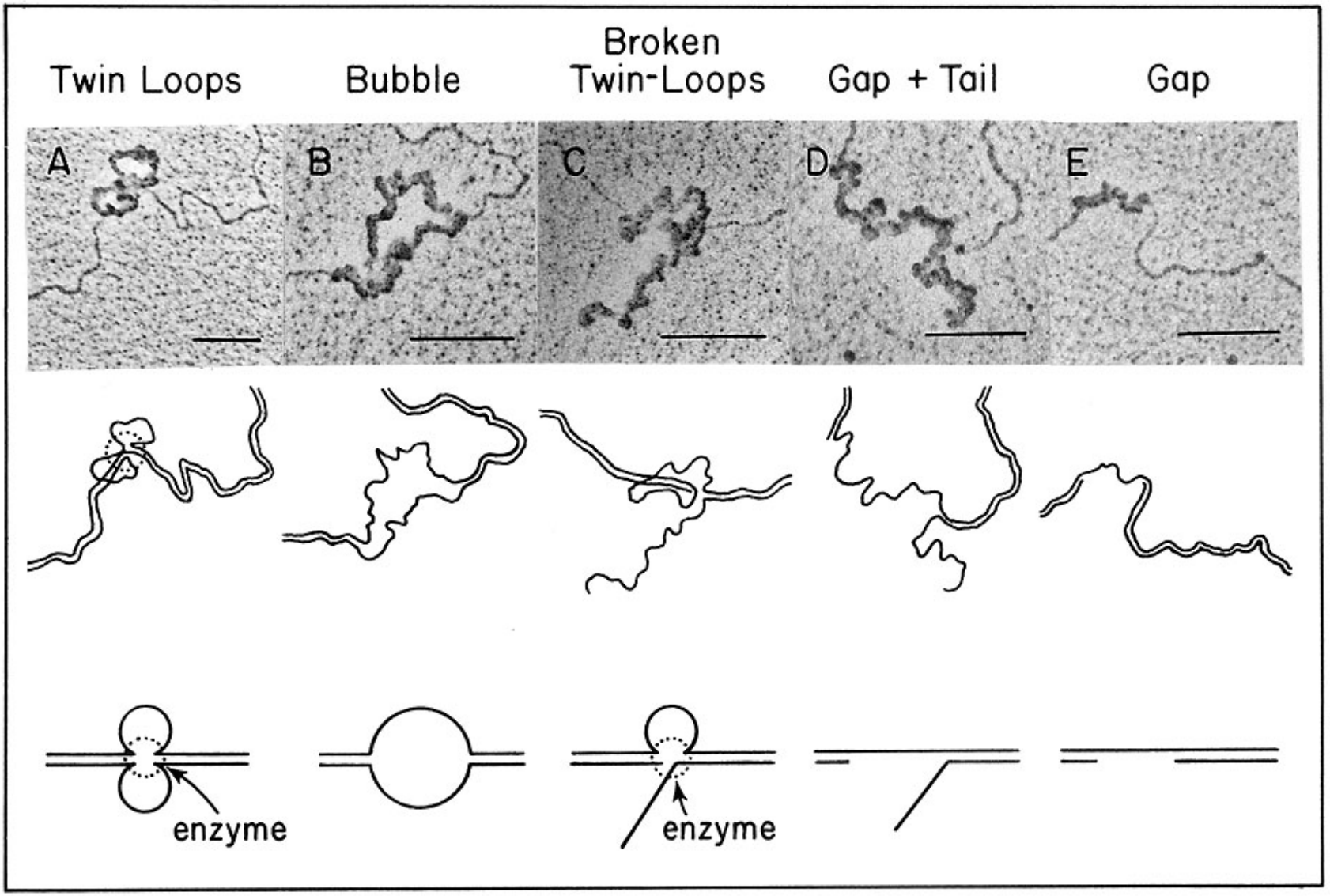
RecBC(D)


Digestion of Duplex DNA by *RecBC* DNase-ATPase
3' or 5'
+
+
+


Tail
Fork
Loop + Tail
Loop + Tails
A
B
C
D
enzyme
enzyme

RecBC(D) action with 1mM Ca^{2+} present




Twin Loops
Bubble
Broken
Twin-Loops
Gap + Tail
Gap
A
B
C
D
E
enzyme
enzyme

Coming of Age with Aging

ALTERED PROPERTIES OF DNA POLYMERASES FROM LATE PASSAGE CULTURED HUMAN FIBROBLASTS

Level of α-, but not β-polymerase reduced.

Species of α-polymerase elute earlier from DEAE-cellulose.

Species of α-, β- and γ-polymerase become less faithful.

EXAMPLES OF MISINCORPORATION

Polymer	Non-homologous Triphosphate	Divalent Cation	Misincorporation Young Enz.	Old Enz.
Poly (dA-dT)	dGTP	Mg^{++}	1/1820	1/470
Poly(dA)·Poly(dT)	dGTP	Mn^{++}	1/730	1/86
	dCTP	Mg^{++}	<1/5600	1/700
Poly (d I-dC)	dATP	Mn^{++}	1/940	1/180
Poly(dI)·Poly(dC)	dATP	Mn^{++}	<1/16,000	1/1700
	dTTP	Mn^{++}	1/11,000	1/3700

DNA POLYMERASE ACTIVITIES FROM CULTURED HUMAN FIBROBLASTS

	mg Protein per g cells	Units Activity per g Cells		
		Unfractionated	Fractionated	
			α Polymerase	β Polymerase
IMR-90				
PDL=21	31	174	54	11
PDL=29	27	57	19	9
PDL=37	25	44	11	9
PDL=40	29	52	12	11
PDL=42	28	26	9	6
PDL=45	22	29	7	3
F65				
Log Phase	19	170	35	18
Butyrate-treated Log Phase	21	115	32	16
Postconfluent	18	35	2.2	11

S. W. Krauss &. S. Linn (1986) *J. Cell Physiol.* **126:** 99-106

EXAMPLES OF MISINCORPORATION - HELA POLYMERASE

	$[PO_4^{-3}]$ eluted from DEAE, Molar	Error-Frequency
Log Phase	0.22 - 0.24	<1/76,000
	0.25 - 0.26	<1/86,000
	0.28 - 0.30	<1/44,000
	β	<1/18,000
Stationary	0.16 - 0.19	1/12,000
	0.20 - 0.23	1/15,000
	0.25 - 0.26	1/26,000
	β	1/4400

Assay with Poly (dA-dT), dGTP as non-homologous dNTP, and $MnCl_2$.

SOME POSSIBLE CAUSAL RELATIONS BETWEEN DNA DAMAGE/REPAIR AND AGING

DNA repair efficacy drops with age (passage)

The rate of DNA damage increases with age (passage)

DNA repair is slightly less effective
than is necessary for the frequency of DNA damage

Certain types of DNA damage are not subject to DNA repair

DNA REPAIR EFFICACY INCLUDES...

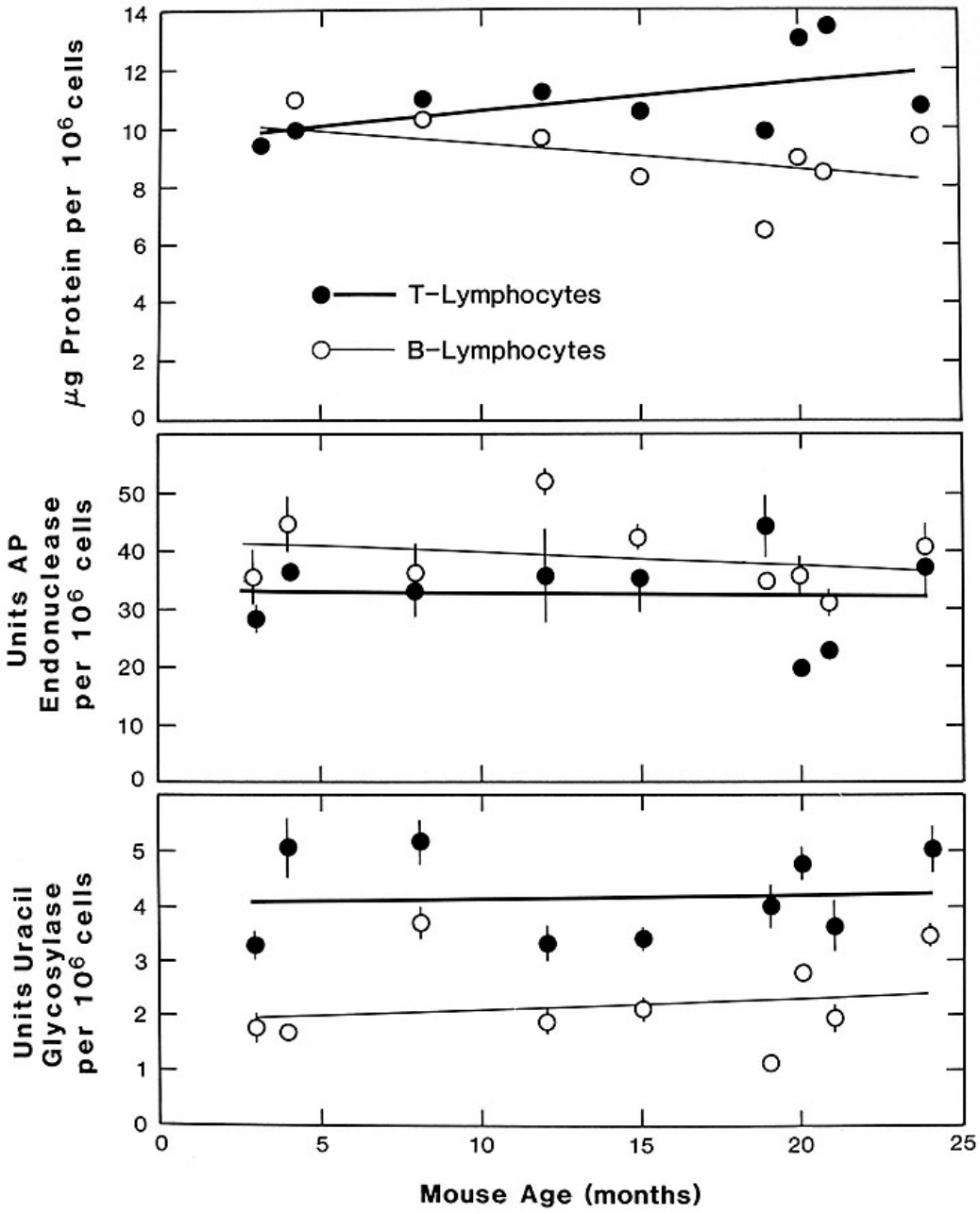
Removal (prevention of accumulation) of damage

Fidelity (avoidance of base substitutions)

Reestablishment of modifications, etc.
(e.g., methylation, regulatory proteins, structural proteins)

Ligation of strand and chromosome breaks

J. Barnard, M. LaBelle, & S. Linn *Exp. Cell. Res.* (1986) **163:** 500-508



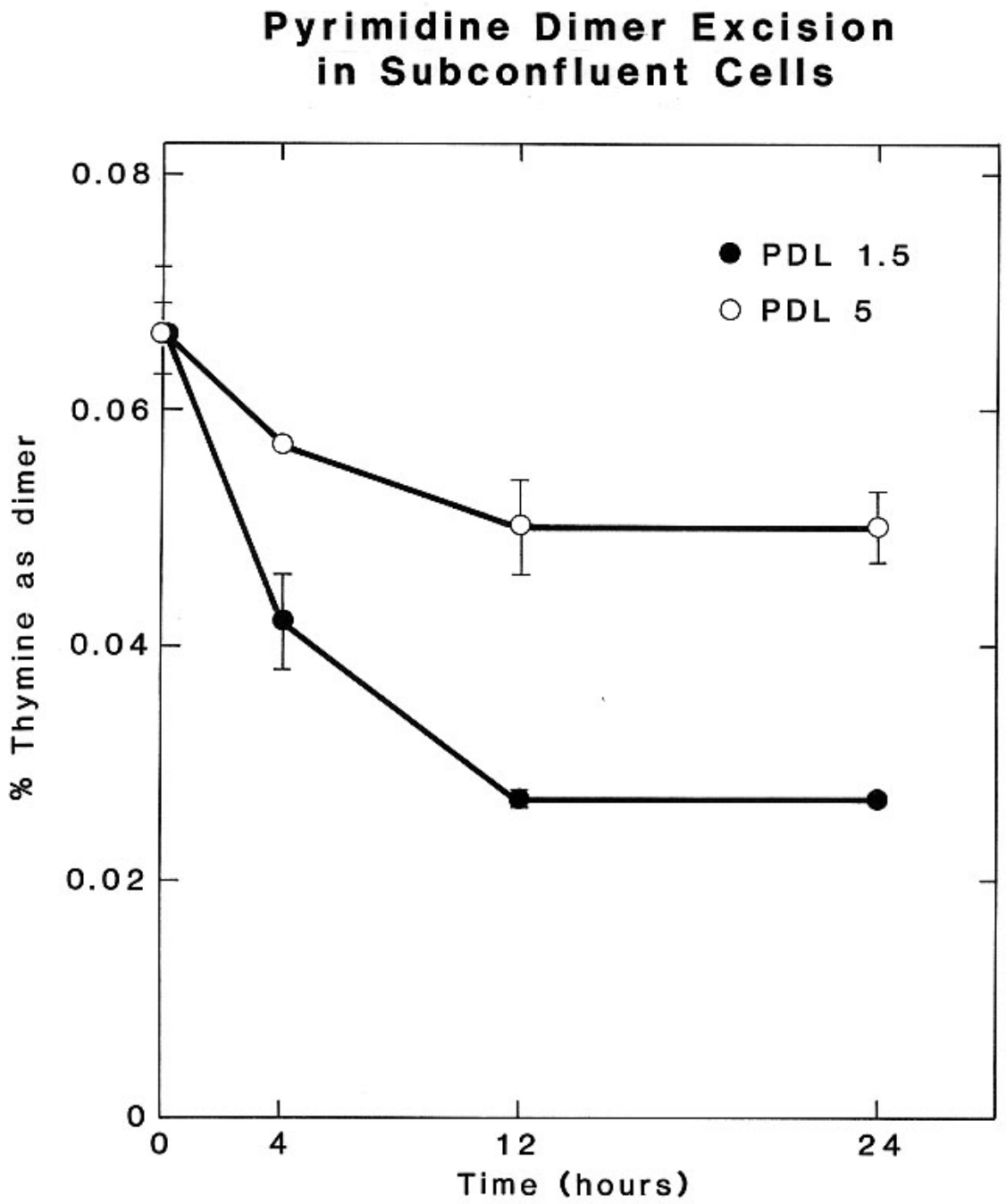
MPC11
(Leukemic B line)

169 μg/10^6 cells

1650 U /10^6 cells

143 U /10^6 cells

Mouse primary skin fibroblasts



M. LaBelle & S. Linn (1984) *Mut. Res.* **132:** 51-61

Aging studies might....

include comparisons of pre-differentiated cells and their terminally differentiated counterpart.

consider the accumulation in somatic, particularly non-mitotic cells of perturbations of chromosomal regulatory and structural elements as well as of DNA sequences and methylation patterns.

DNA REPAIR PARAMETERS
OF NGF-TREATED NEUROBLASTOMA LINE SH-SY5Y

UV-induced unscheduled DNA synthesis drops to <10%

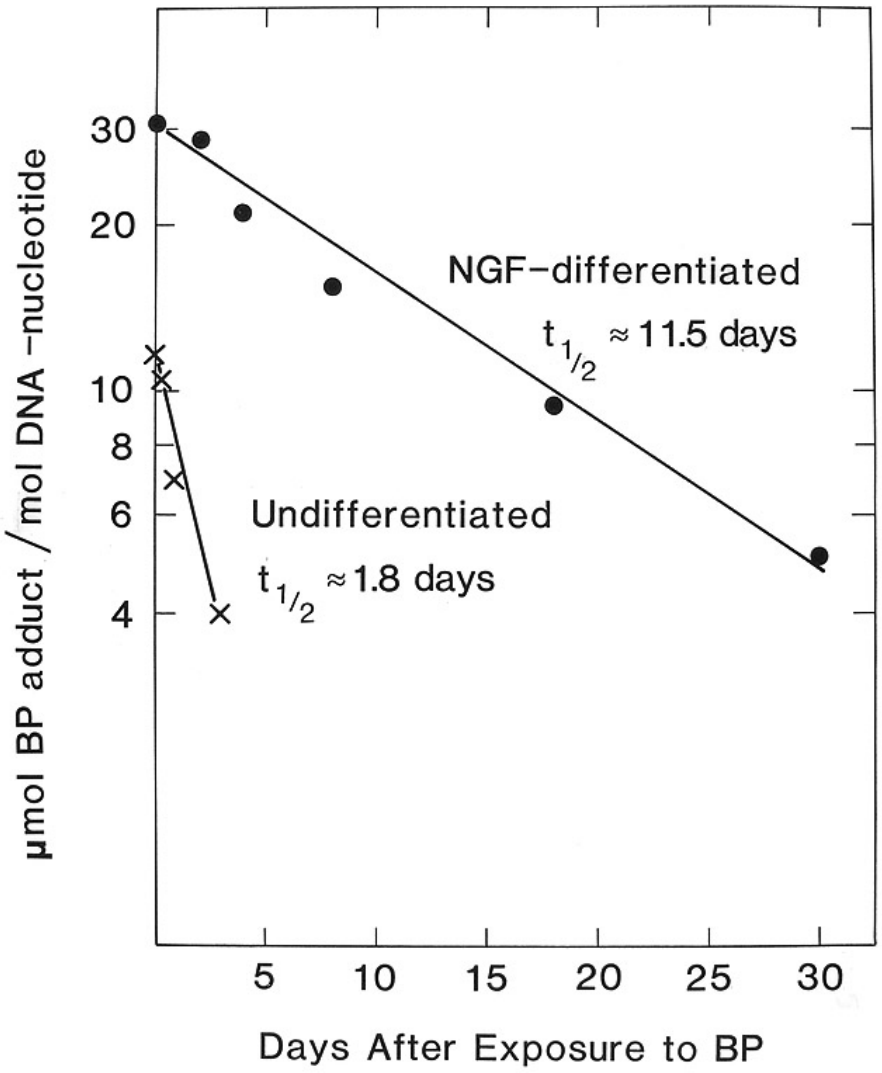
Rate of removal of Benzo[a]pyrene DNA adducts decreased to 15%

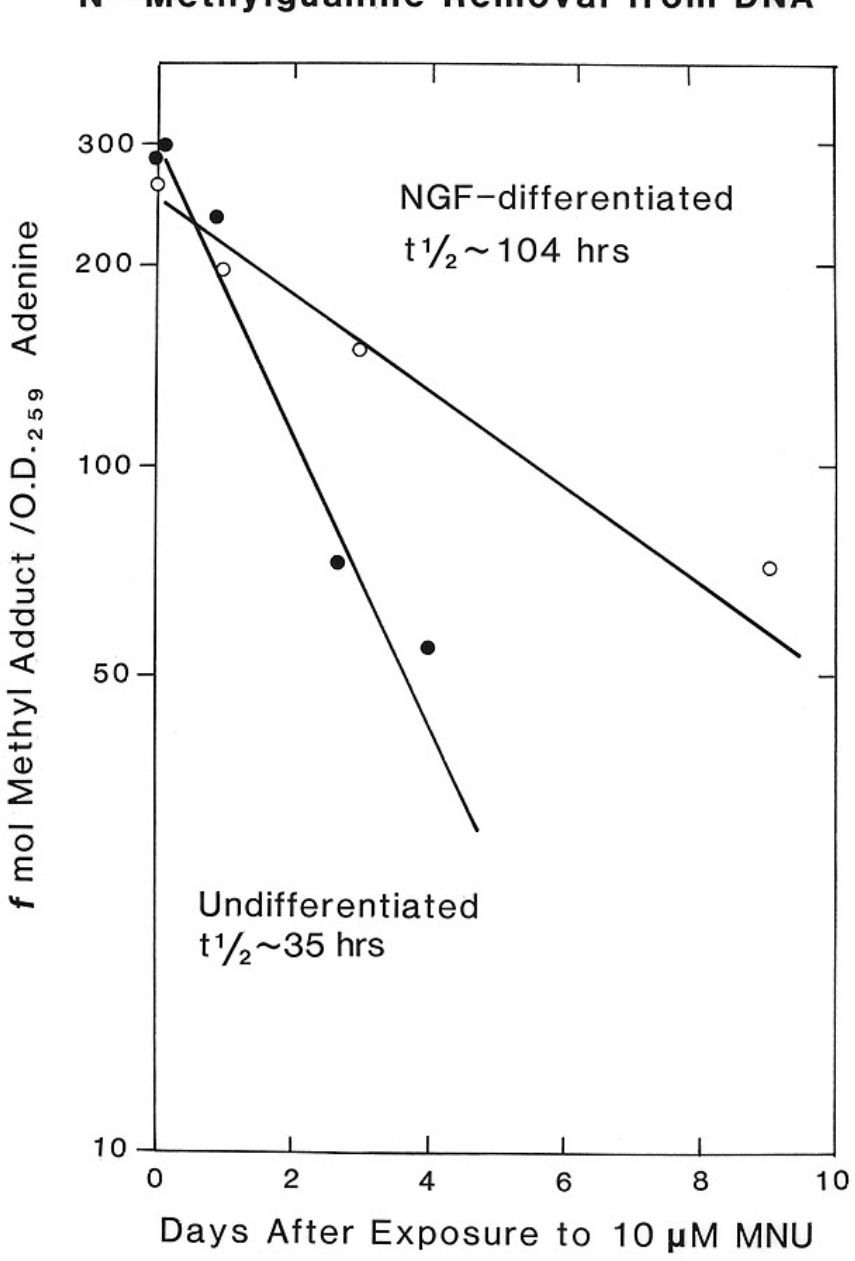
Rate of removal of Benzo[a]pyrene-diolepoxide
DNA adducts decreased to 19%

Rate of removal of methylated purines 50% - 100%,
except for 7-meG which is reduced 3-fold to the spontaneous rate.

L. M. Jensen & S. Linn (1988) *Mol. Cell Biol.* **8**: 3964-3968

BENZO[a]PYRENE DNA ADDUCT REMOVAL




N7-Methylguanine Removal from DNA
300
200
100
50
10
f mol Methyl Adduct /O.D.259 Adenine
NGF-differentiated
t1/2~104 hrs
Undifferentiated
t1/2~35 hrs
0
2
4
6
8
10
Days After Exposure to 10 µM MNU

"DNA REPAIR" ENZYMES IN NGF-TREATED NEUROBLASTOMA LINE SH-SY5Y COMPARED TO UNTREATED CELLS

	Units of Enzyme/10^6 cells
DNA polymerase α	20%
DNA polymerase β	unchanged
Total AP endonuclease	280%
AP endonuclease I	240%
Uracil DNA glycosylase	unchanged
DNA Methylase	40%

Mitochondrial DNA Damage and Aging

Do damaged mitochondrial DNA nucleotides accumulate with age?

Do mitochondrial DNA damages (including base changes, duplications, deletions, etc.) accumulate with age?

Do mitochondria repair genomes or eliminate damaged genomes?

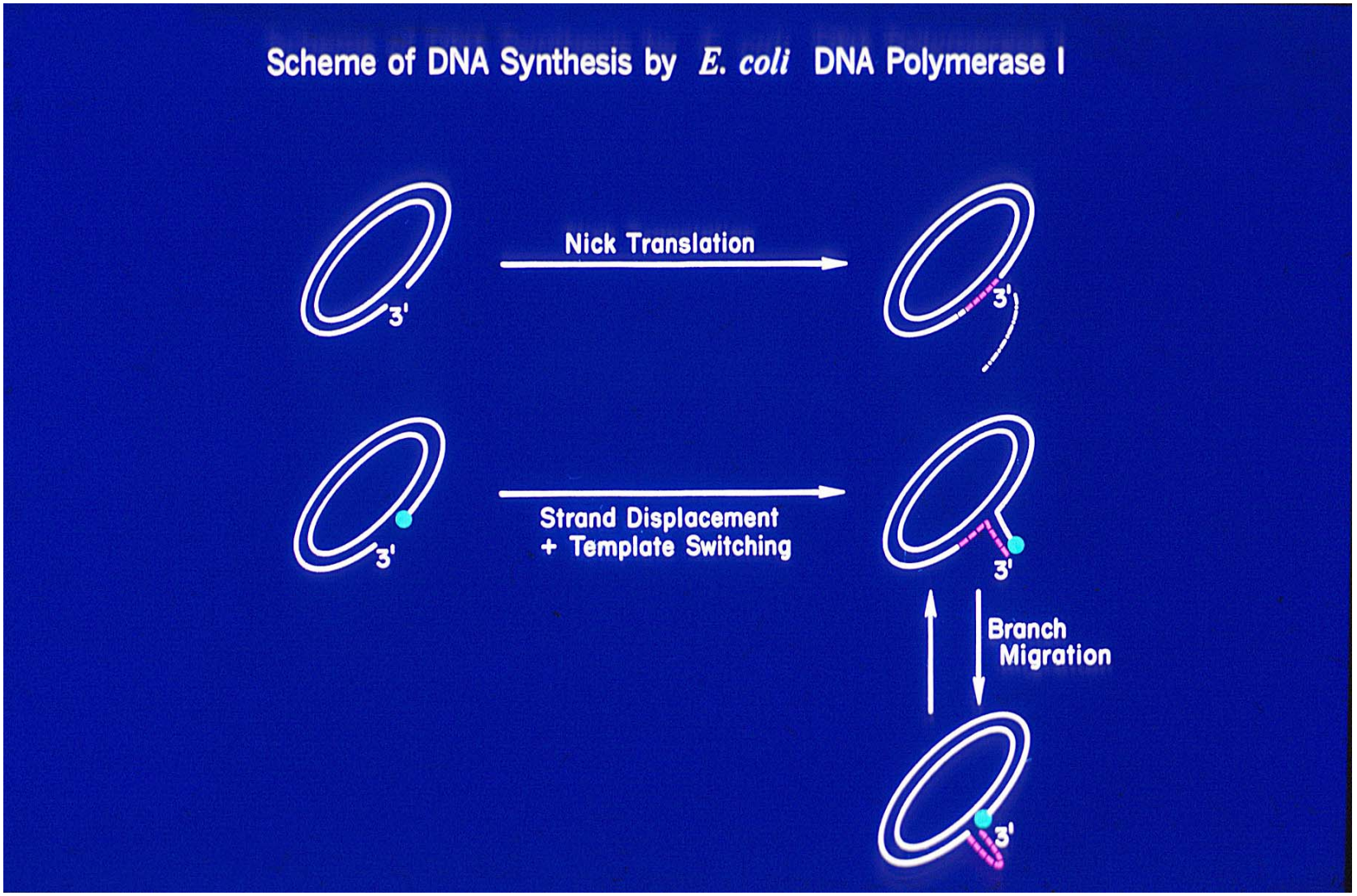
Does the accumulation of damaged genomes in the mitochondria contribute to an aging error catastrophe?

DNA polymerases

"The Kornberg Enzyme (*E. coli* pol I) is probably *just* a repair enzyme"

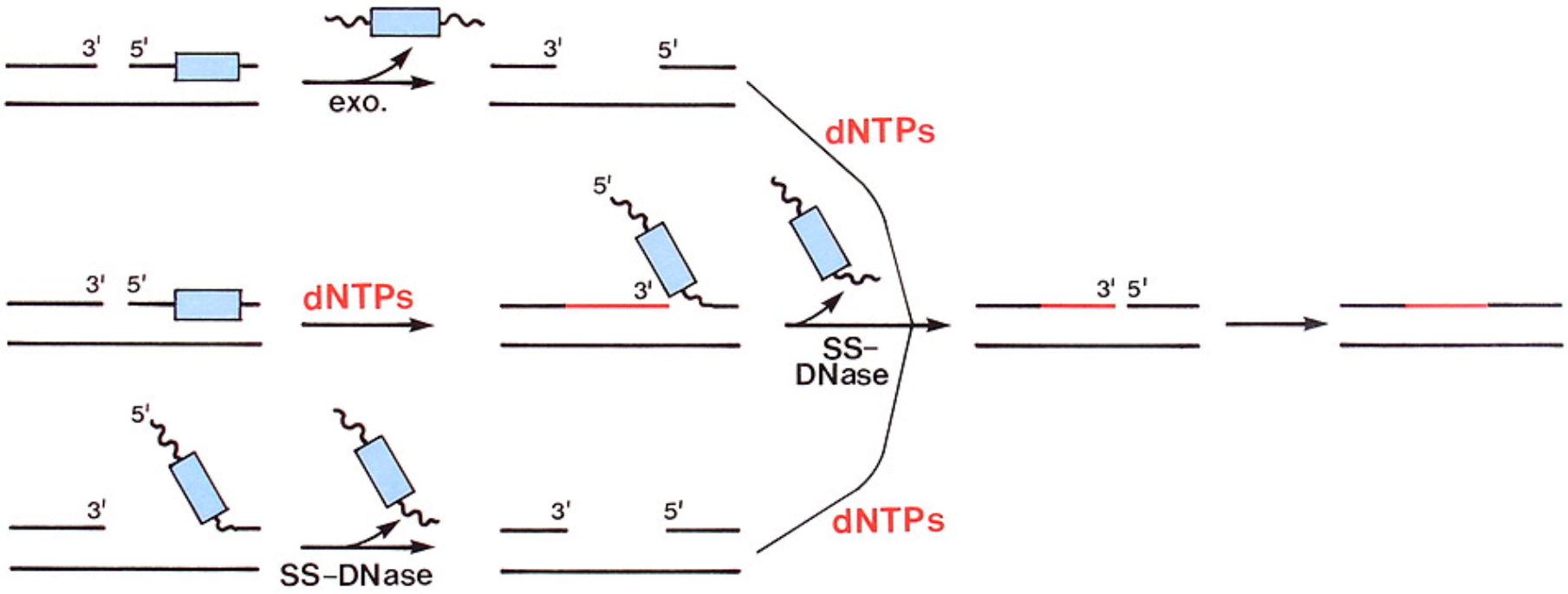
--Mark Bretcher, Stanford 1967

(prior to the report of the *polA* mutant by De Lucia and Cairns in 1969)



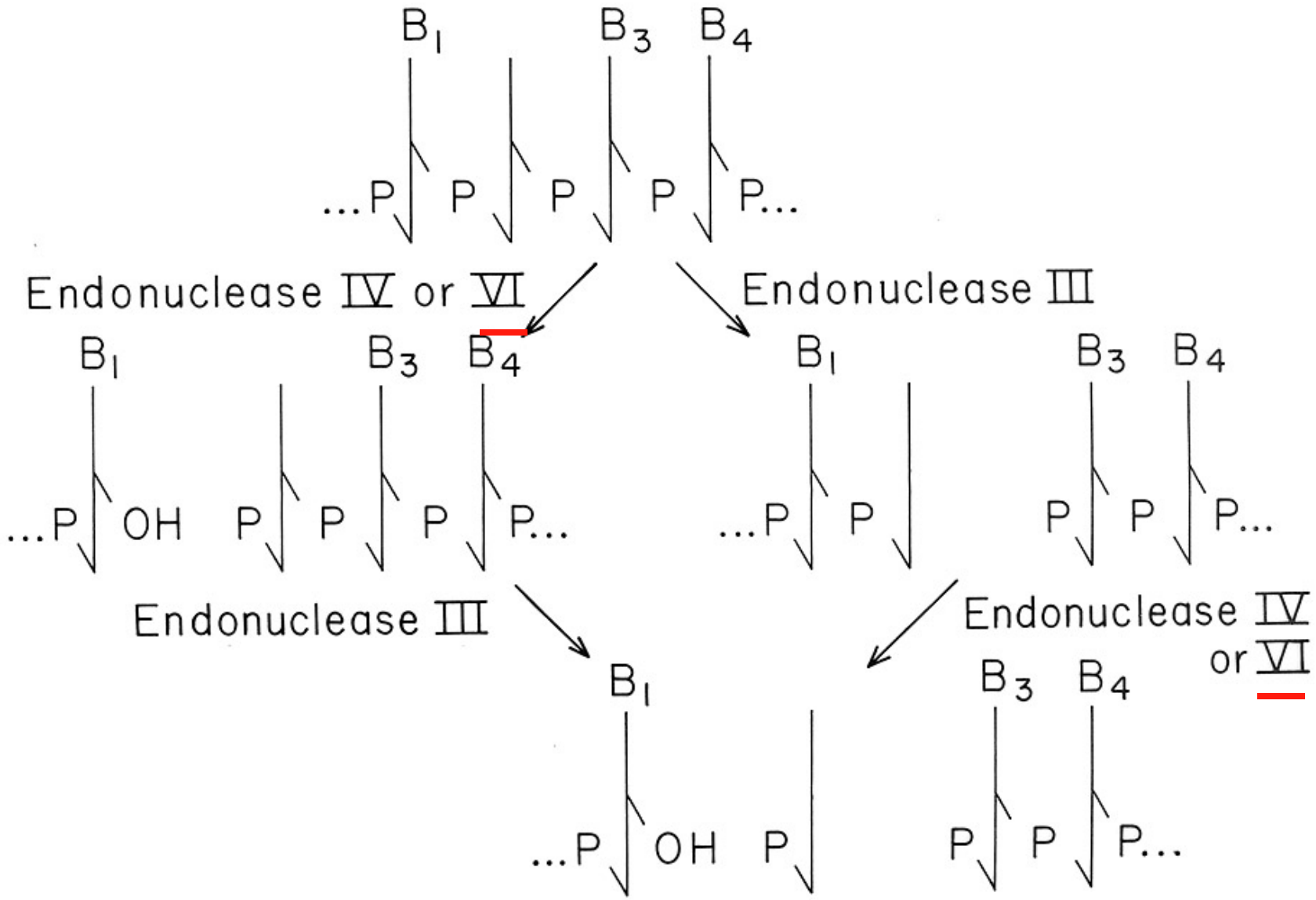
Mosbaugh & Linn **JBC 257:**575 (1982)

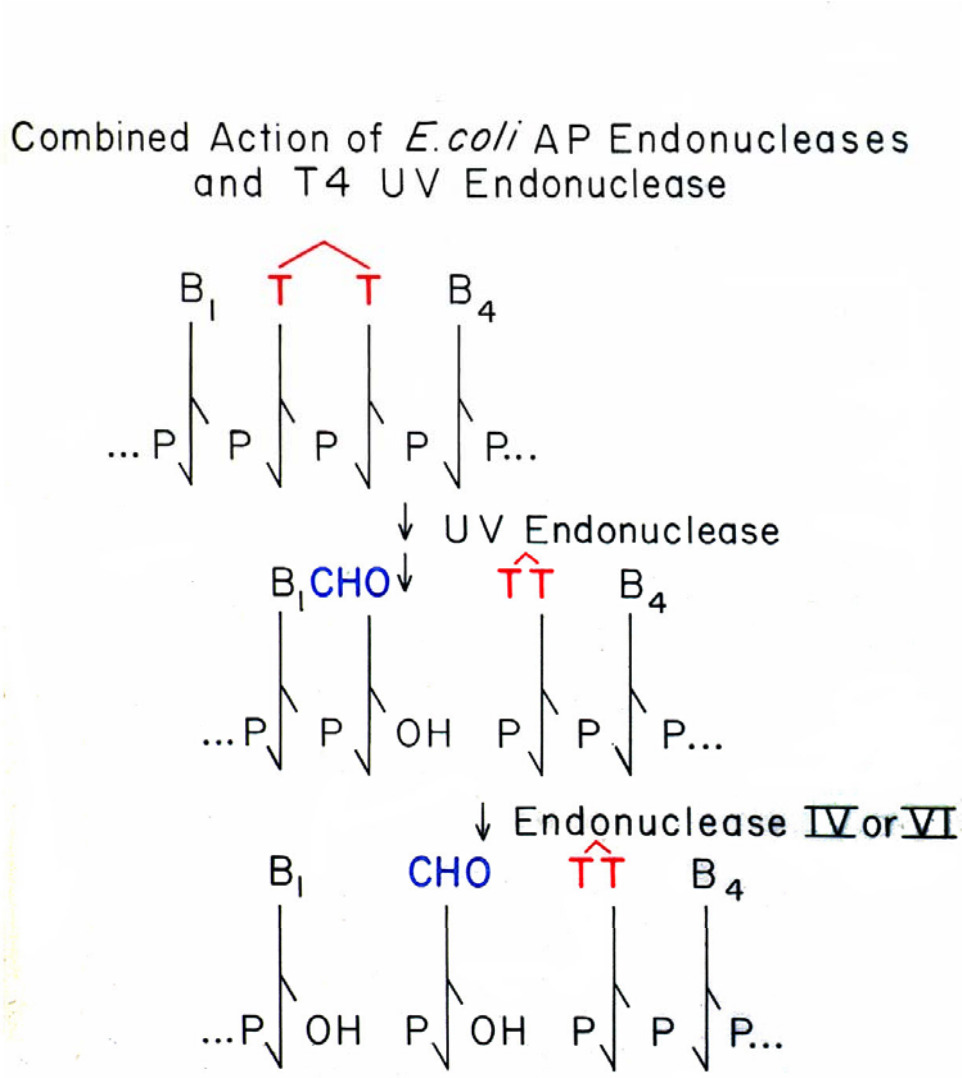
POSSIBLE MODES OF EXCISION + REPAIR SYNTHESIS



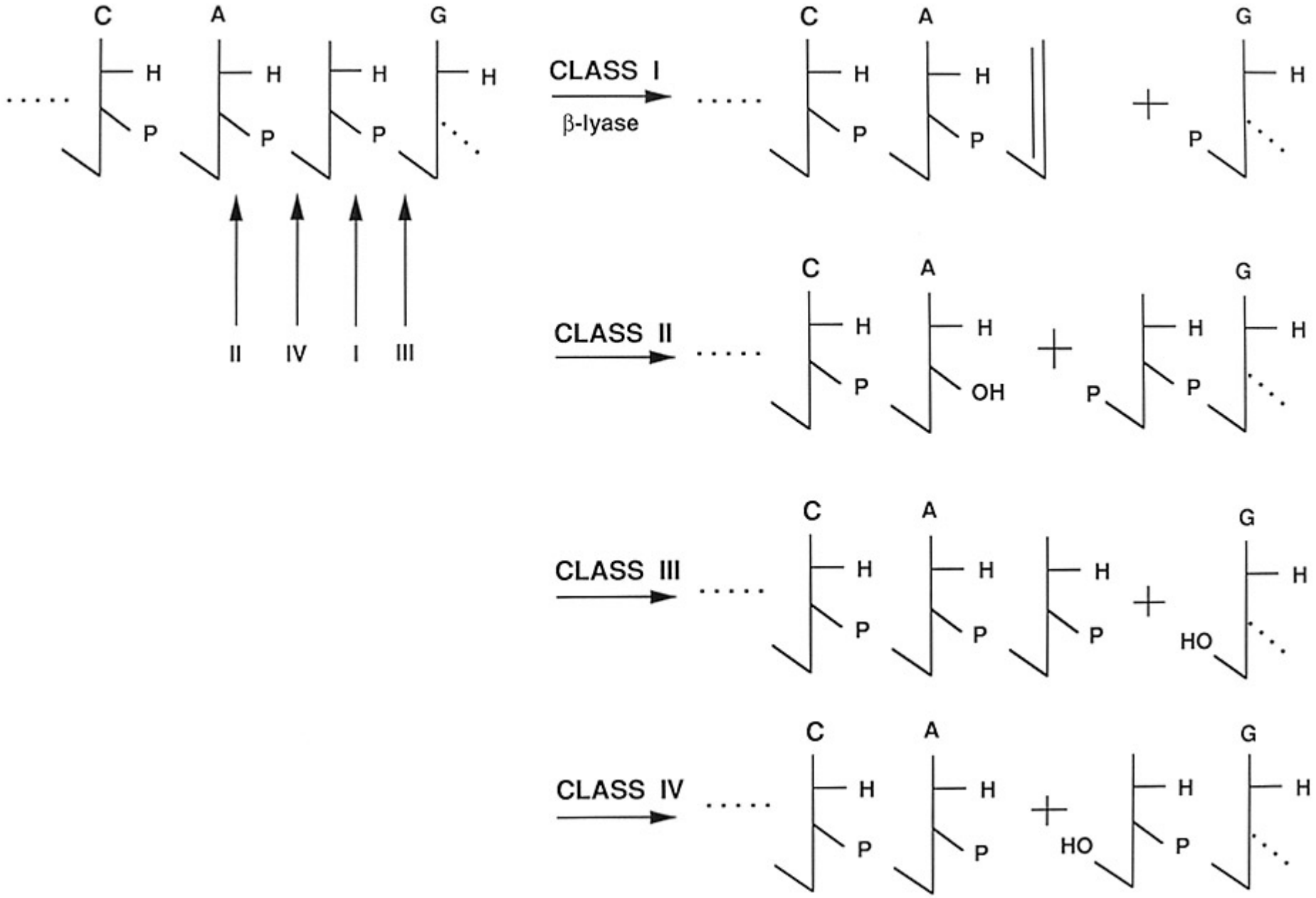
AP and “UV” Endonucleases

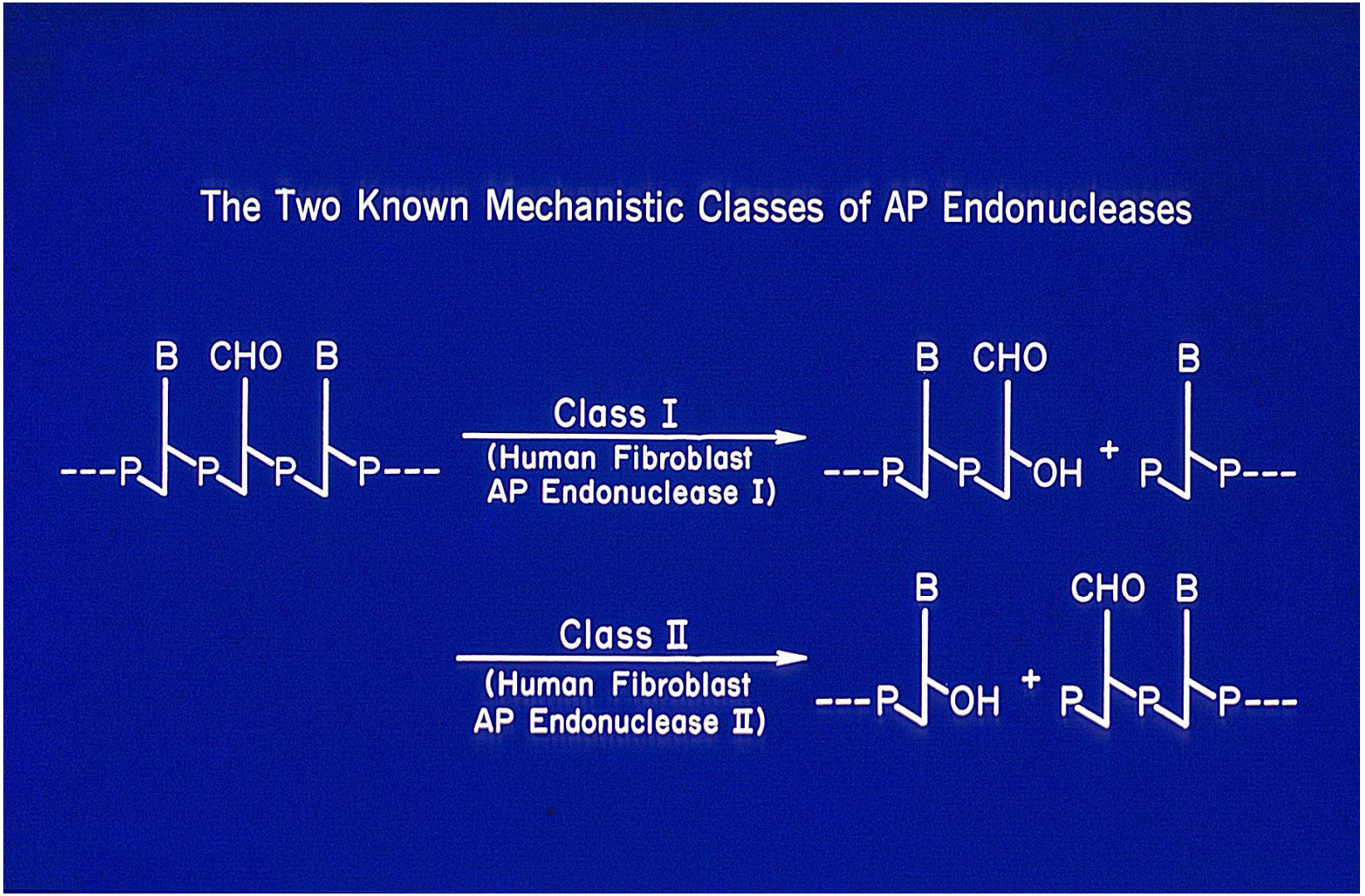
Combined Action of *E. coli* AP Endonucleases





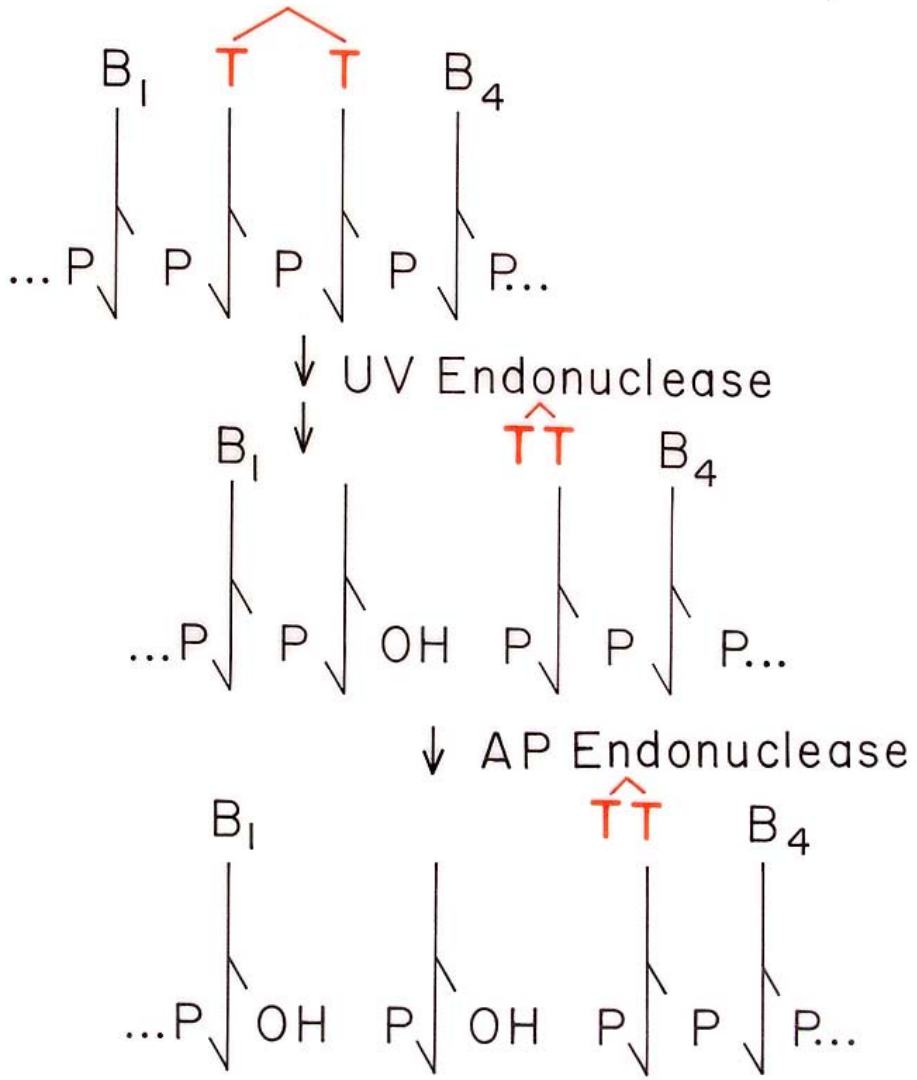
Mosbaugh & Linn **JBC 258:**108 (1983)


C
A
G
H
P
II
IV
I
III
CLASS I
β-lyase
CLASS II
OH
CLASS III
HO
CLASS IV
HO

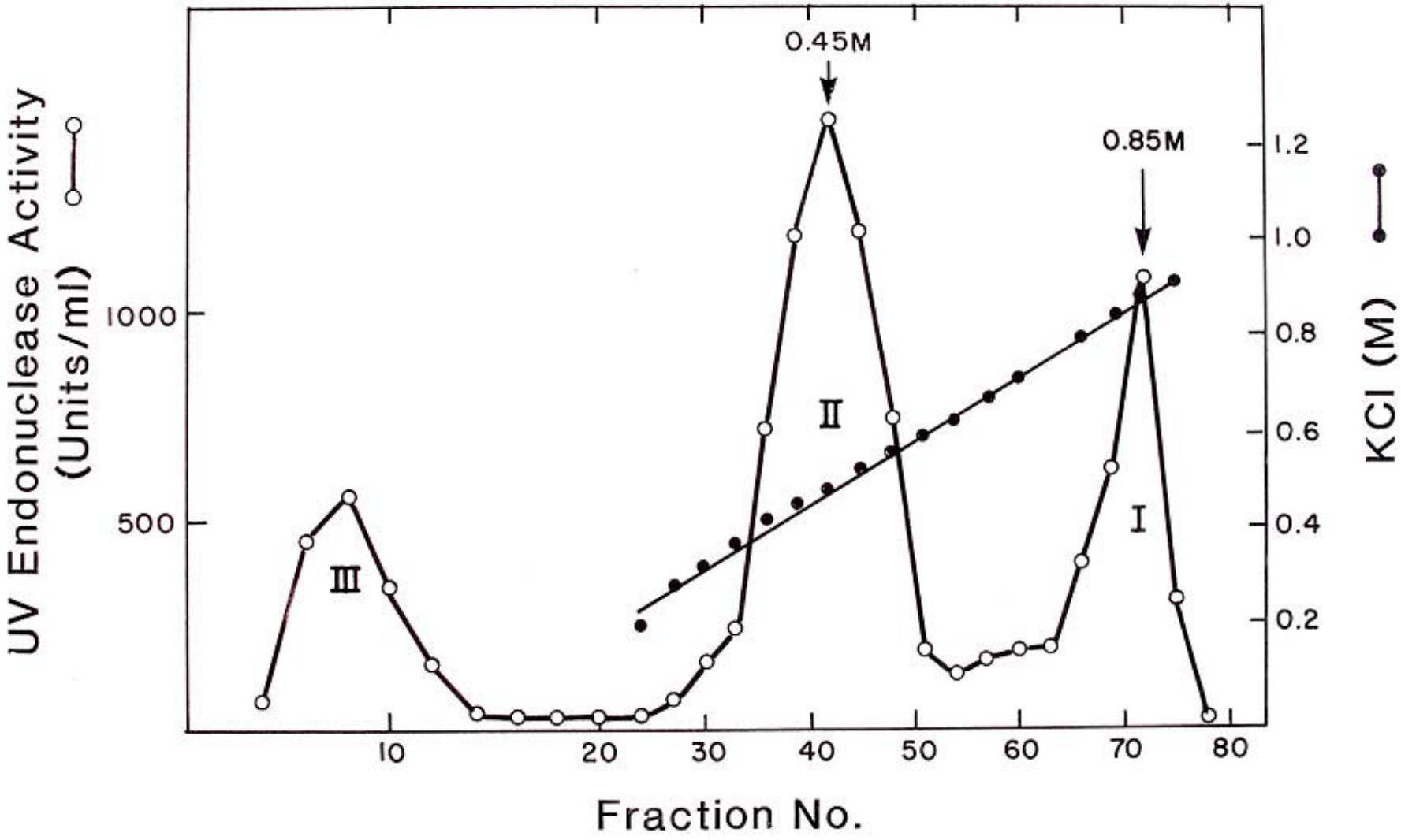


Mosbaugh & Linn **JBC 255**:11743 (1980)

Proposed Combined Action of Human AP Endonuclease and T4 UV Endonuclease



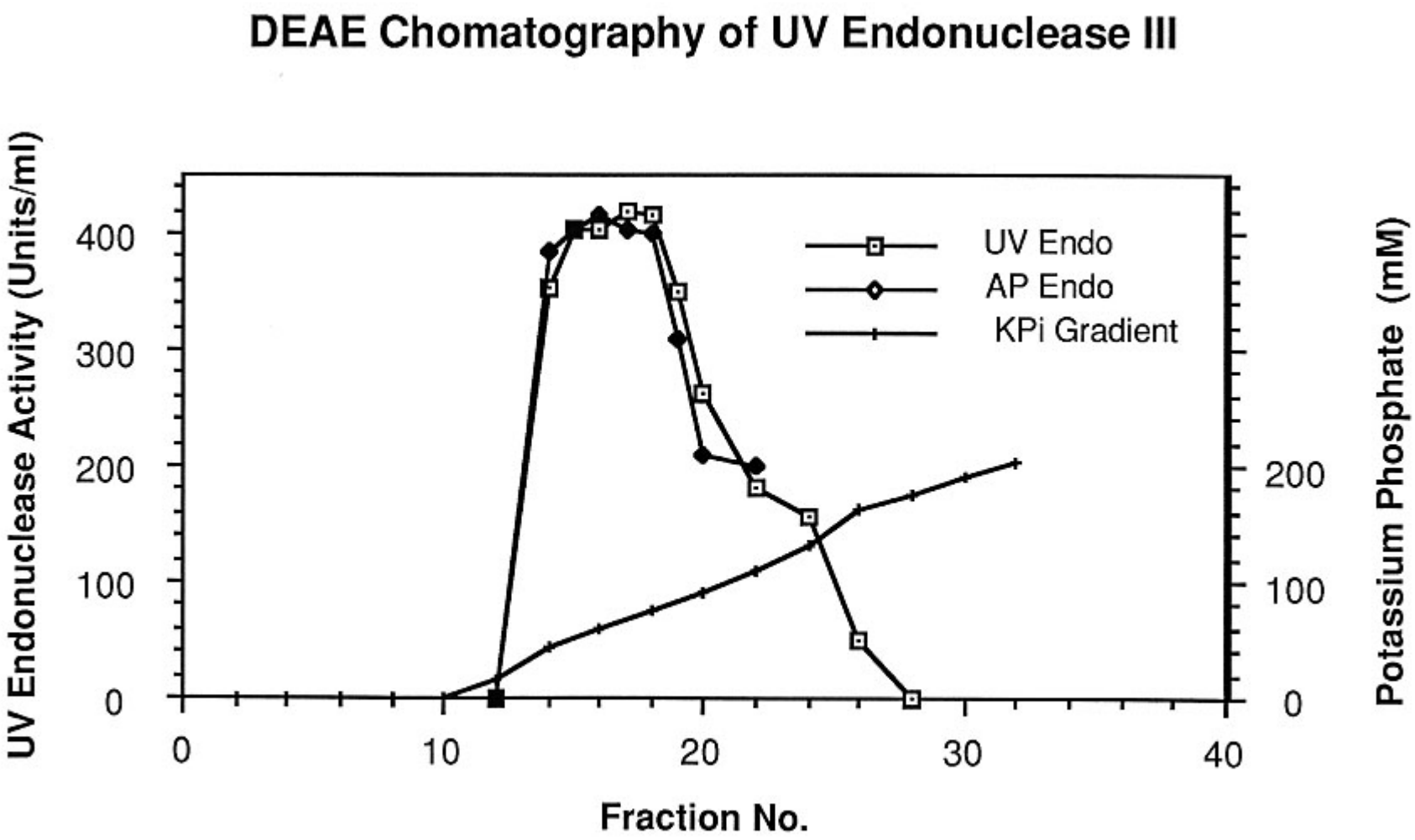
Phosphocellulose Column Chromatography of UV Endonucleases



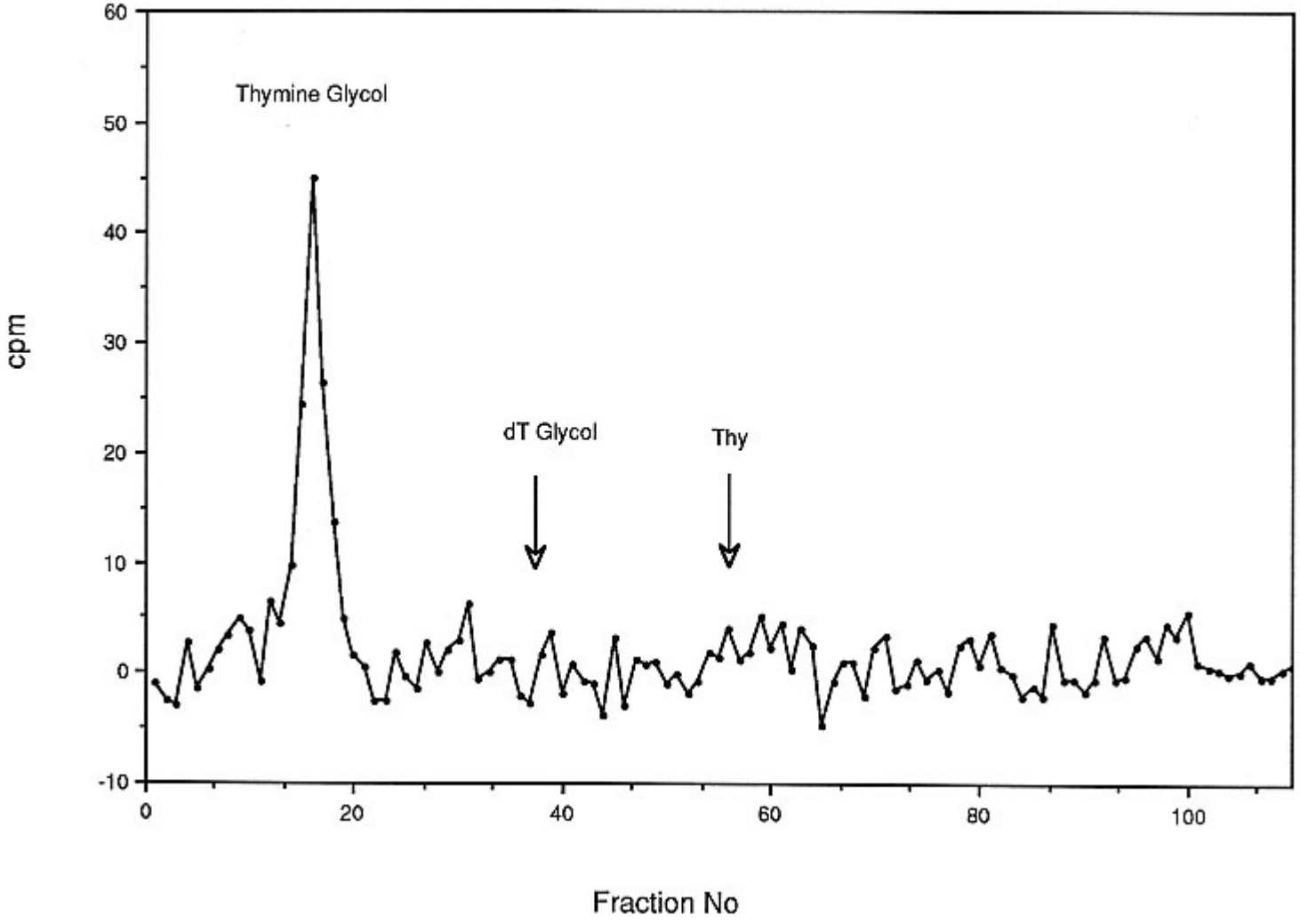
Purification of UV Endonuclease III

	Vol	mg Protein	Units Activity	U/mg
Crude	300	15,000	(2,400,000)	(160)
DEAE-I	375	11,300	(3,900,000)	(350)
P-Cell	250	3000	300,000	100
DEAE-II	164	330	210,000	630
$AmSO_4$	8	181	120,000	640
Sephacryl S-200	17.2	4.8	12,000	2,500
Hydroxylapatite	2.0	0.5	3,800	7,600
Heparin Agarose	0.5	0.077	1,660	21,500
Sucrose Gradient	4.0	0.032	1,020	32,000
peak		0.006	310	52,000

Purification from 96-liters (1.1×10^{11} cells) of MPC-11 murine plasmacytoma culture.


DEAE Chomatography of UV Endonuclease III
UV Endonuclease Activity (Units/ml)
400
300
200
100
0
0
10
20
30
40
Fraction No.
UV Endo
AP Endo
KPi Gradient
200
100
0
Potassium Phosphate (mM)

Action of UV Endonuclease III on OsO4-treated [3H-thy] DNA



THREE AMINO ACID SEQUENCES OF THE UV ENDONUCLEASE III ARE IDENTICAL TO THOSE OF THE RAT RIBOSOMAL PROTEIN S3.

UV III Sequences

1.	(6 AA) :	KRFGFP
2.	(16 AA) :	KVATRGLCAIAQAESL
3.	(14 AA):	KGGKPEPPAMPQPV

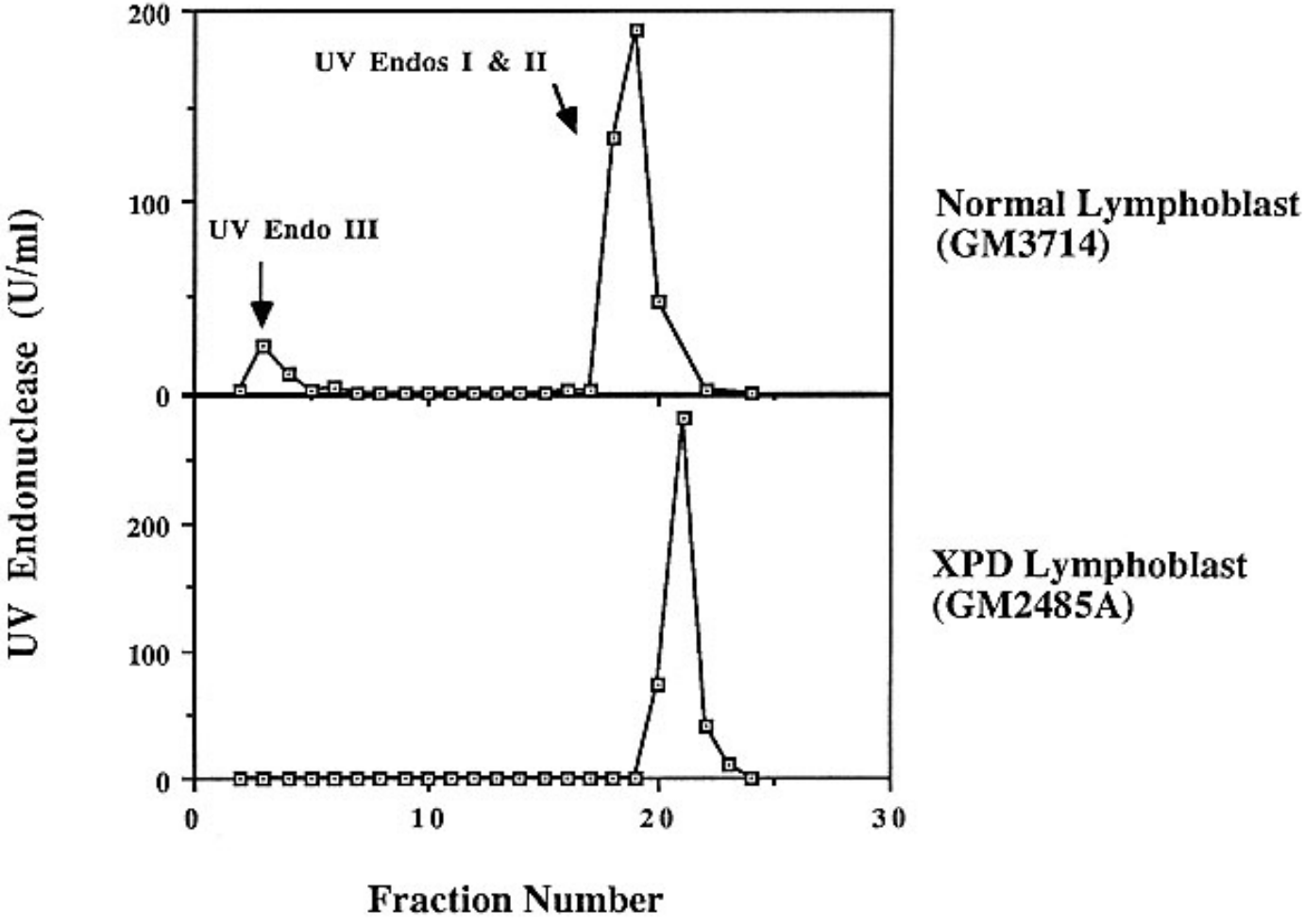
Three sequences determined by Arie Admon are respectively identical to the rat ribosomal S3 sequences described by Ira G. Wool 75-80, 90-105, and 227-240 (Arch. Biochem. Biophys. 1991,. 283 546-550)

The amino terminal amino-acid sequence of the UV endonuclease III is blocked as is the N terminal sequence of S3 .

Both the protein and UV/AP endonucleases are immunodepleted by abs. against rat rpS3 and rat rpS3 produced in *E. coli* has the two activities.

J. Kim, et al. (1995) *J. Biol. Chem.*: **270: 13620-13629**

Phosphocellulose Chromatography of Cell-free Extracts from Human Lymphoblasts



DISTRIBUTION OF ENDONUCLEASE UPON PHOSPHOCELLULOSE CHROMATOGRAPHY

Cell Line	Genotype	Percent Recovered Activity in Flow-Thru
424	normal	13
CRL 1262	normal	18
CRL 1343	ataxia telangiectasia	17
XP 12BE	XP-A	18
XP 25RO	XP-A	26
XP 7BE	XP-D	~ 1
424	normal	26
XP 5BE	XP-D	< 0.5
XP 6BE	XP-D	< 0.5
HeLa	transformed	21

Inactivation of UV Endonuclease III with Calf Intestinal Alkaline Phosphatase

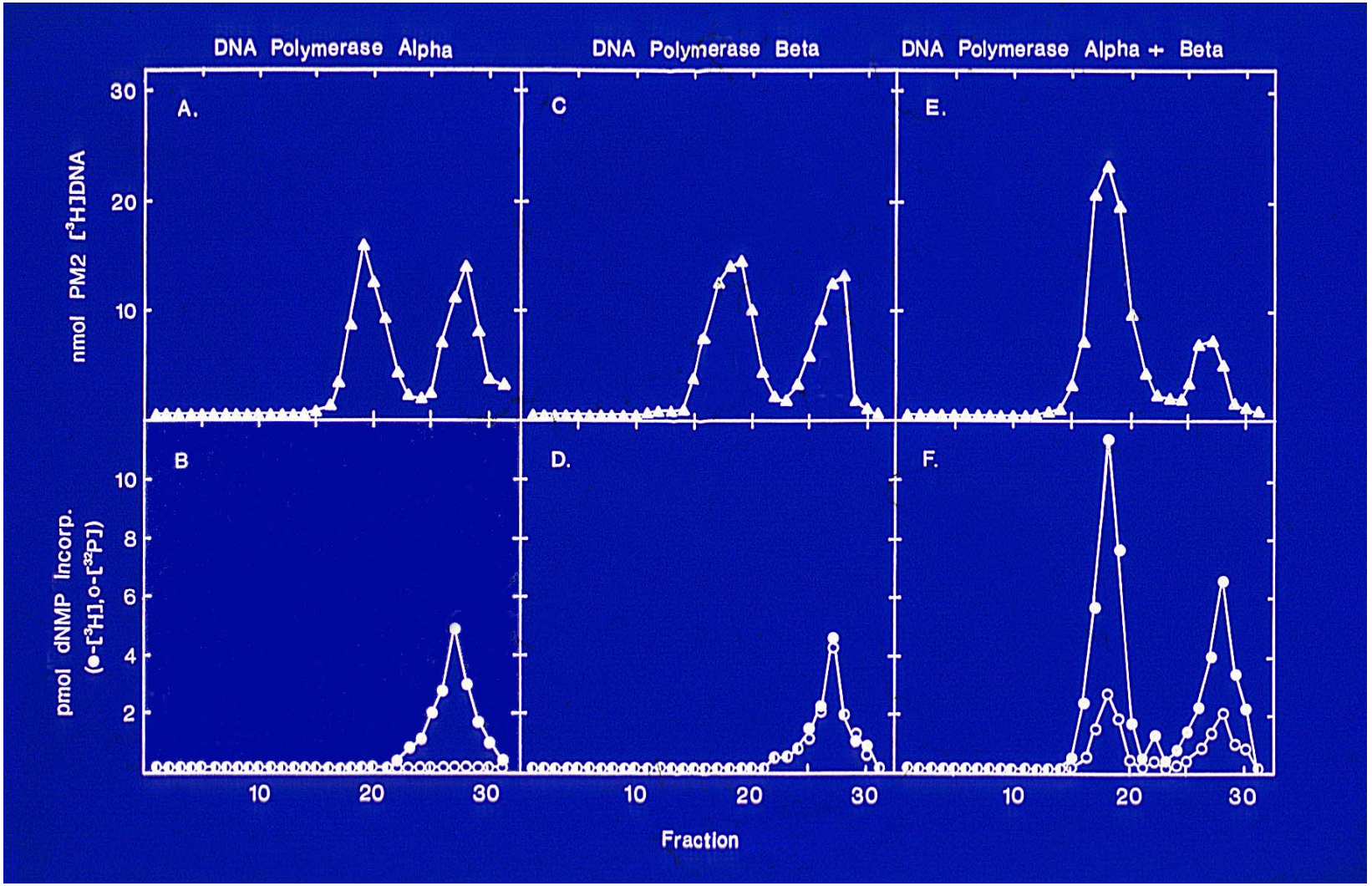
Endo III (0.3 U)	AP (U)	fmol nicks + UV	fmol nicks - UV
-	15	<3	<3
+	0	42	33
+	5	34	26
+	10	7	3
+	15	<3	<3

The Human DNA Polymerases

The good old days…Pols α, β, γ

And then there were five…Pols α, β, γ, δ, ε

And then came the "sloppier copiers"… We're up to ≈17 eukaryotic pols and still counting (and learning the Greek alphabet.)



Mosbaugh & Linn **JBC 259:**10247 (1984)

Gap: 64 dNMP

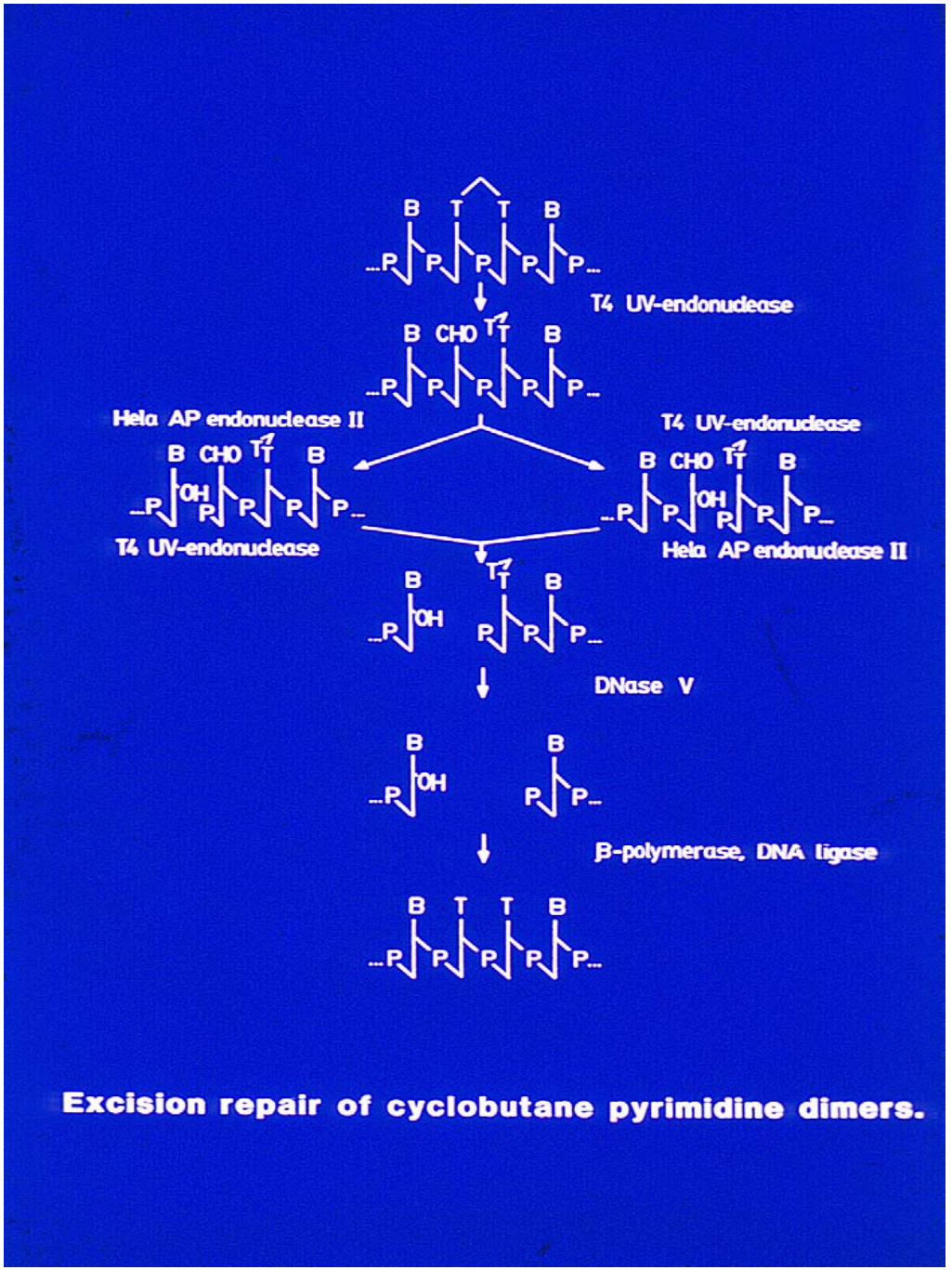
Pol α incorp.: 48 dNMP/gap

Pol β incorp.: 15 dNMP/gap

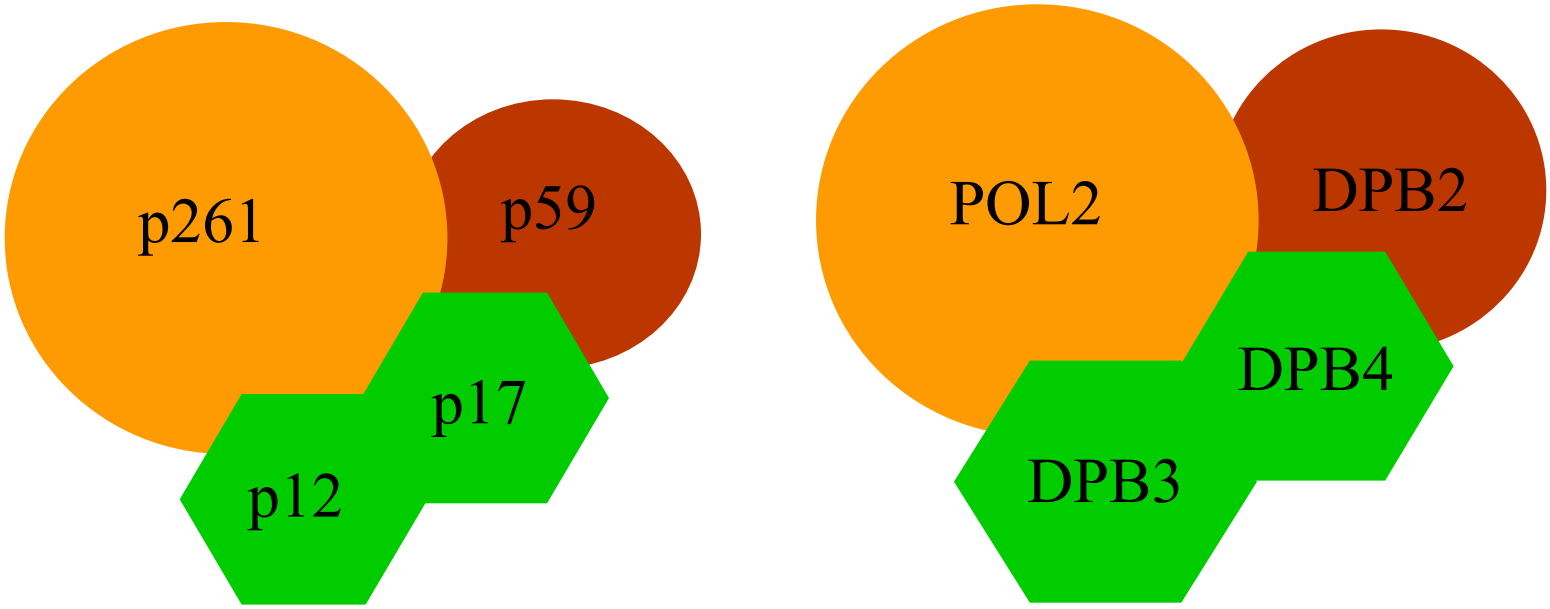
Mosbaugh, Evans & Linn (1984) *CSHSQB* **49:**581

D. H. Evans & S. Linn (1984) *JBC*: **259:** 10252

SV40 chromatin substrate



Pol ε* subunit structure in humans and budding yeast

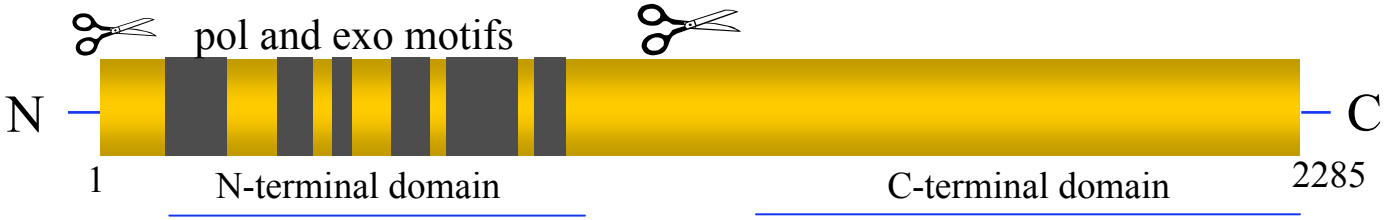


Human

Yeast

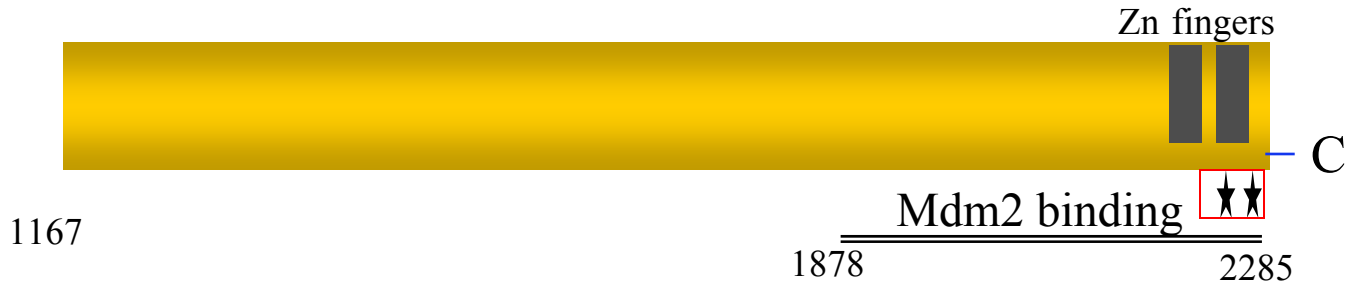
Mammalian pol ε was originally designated as pol δ_2, pol δ_{II} or pol δ

Catalytic subunit - p261



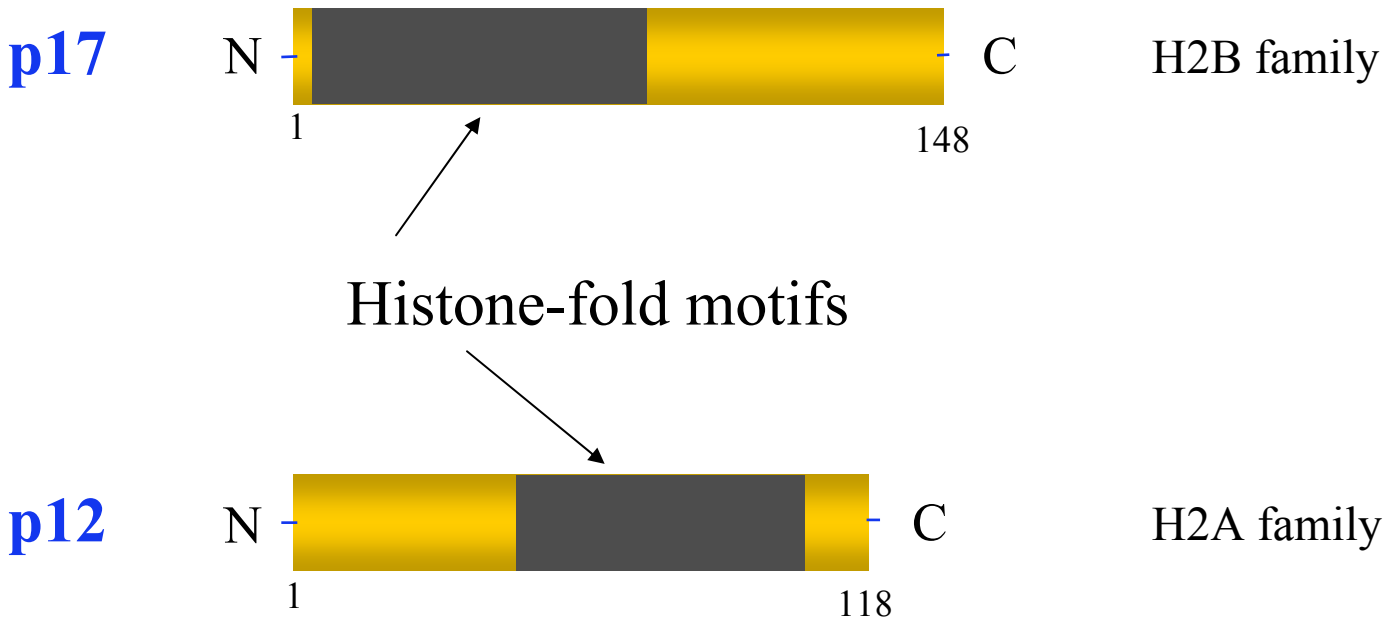
- Polymerase and exonuclease motifs found in the N-terminal domain
- Large C-terminal domain important for protein-protein interactions
- N- and C- terminal domains are separated by a protease-sensitive site. A far N-terminal domain is also separated by a protease-sensitive site. Both sites are cleaved by Caspase 3 during apoptosis.

p261 C-terminal domain



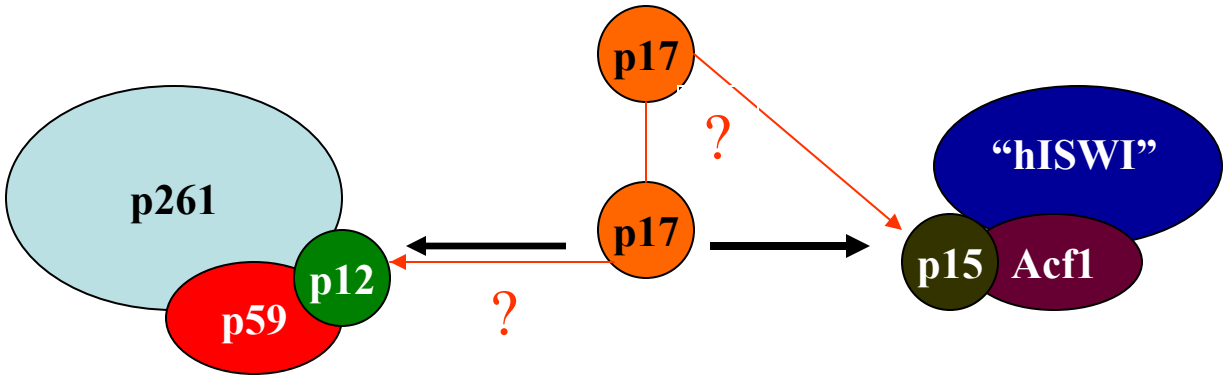
- Contains two zinc fingers
- Necessary for protein-protein interactions
  - Mdm2 binding and stimulation
  - PCNA binding
  - Subunit binding
- ** Necessary to sense replication blocks and delay entry into mitosis in budding, *not* fission yeast
- Essential in both fission and budding yeast

p17 and p12 subunits



pol ε p17 is identical to huCHRAC p17

- CHRAC is a CHRomatin Accessibility Complex first isolated in *Drosophila* but conserved in humans
- Remodels chromatin in an ATP-dependent manner
- huCHRAC contains ACF1 which may target the complex to heterochromatin
- H2A histone-fold motif binding partner of huCHRAC p17 is not pol ε p12, but huCHRAC p15 *Drosophila* similarly has distinct partners for p17.



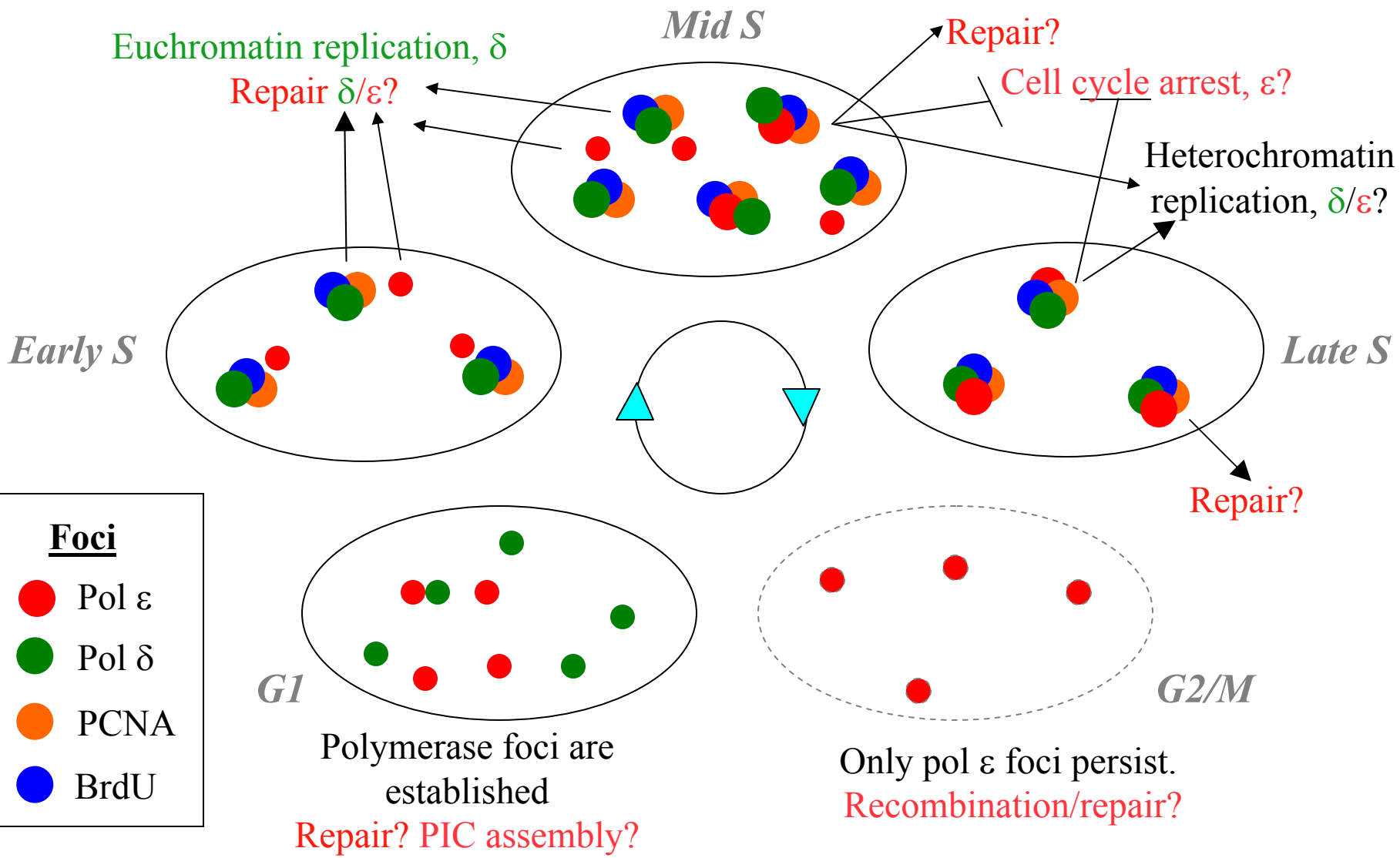
Is there a pol ε : CHRAC complex?

Apparently Not

In *S. cerevisiae*, pol ε replicates telomeres with maintenance of telomer-position effect epistatic states of the Sir complex. But ISW2/yCHRAC binding at telomers promotes reversible switching between epigenetic states.

Ida & Araki, **MCB 24:**217 (2004)

Foci appearance and some putative functions of mammalian pols epsilon and delta during the cell cycle



Outstanding questions

- What is/are the role(s) of the small pol ε foci?
- What is the significance of the sharing of p17 between CHRAC and pol ε?
- Is pol ε a repair and/or a replicative polymerase?
- Where do the error-prone pols localize during the cell cycle before and after DNA damage?

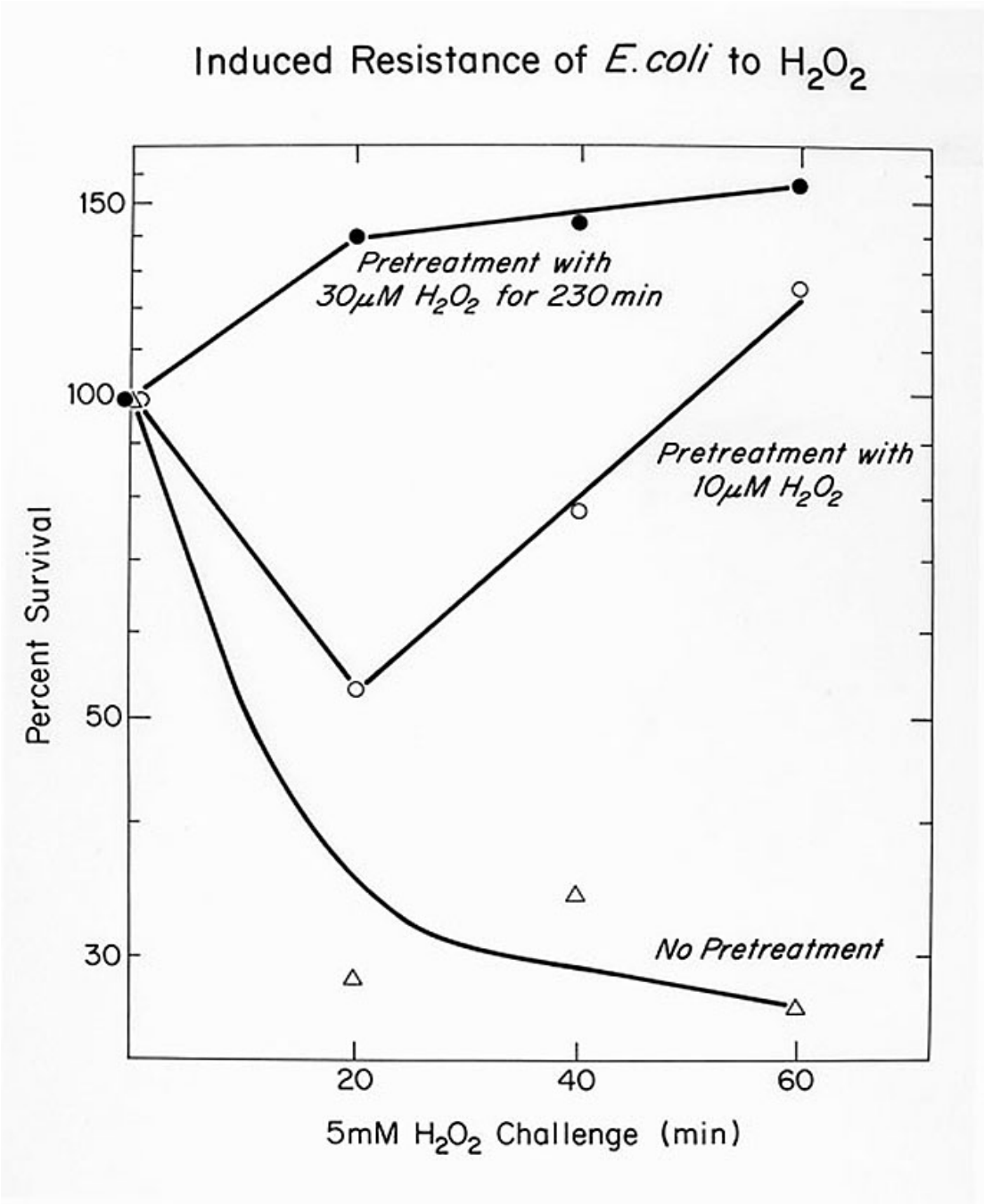
Oxidative DNA Damage

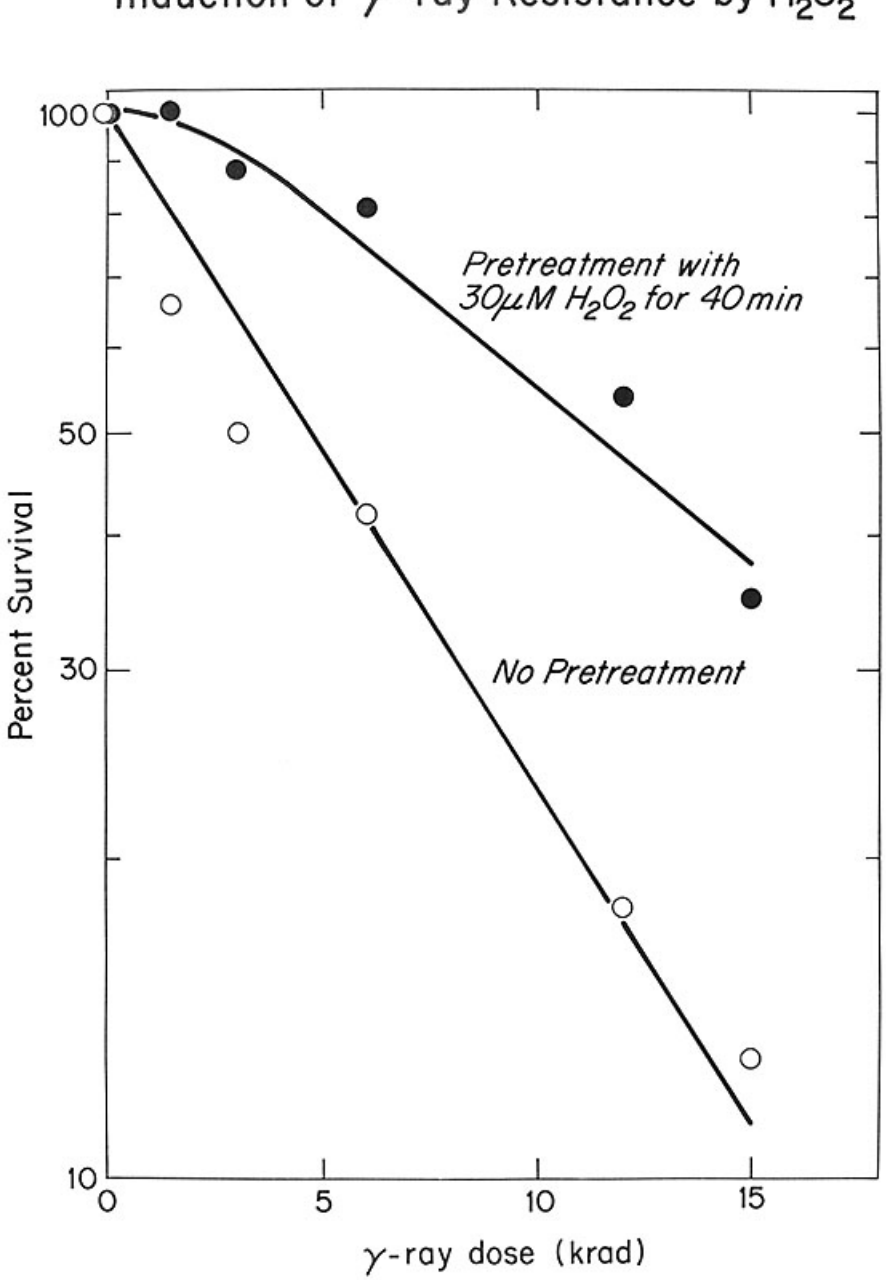
Stress responses

Reactive Oxygen Species (ROS)

Fenton chemistry

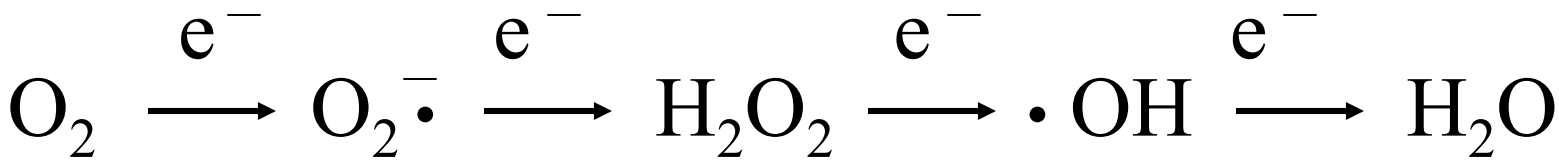
B. Demple & J. Halbrook
Nature (1983)
304: 466-468




Induction of γ-ray Resistance by H_2O_2
100
50
30
10
Percent Survival
Pretreatment with
30μM H_2O_2 for 40min
No Pretreatment
0
5
10
15
γ-ray dose (krad)

Responses to oxidative stress in *E. coli* and genes relevant to DNA repair

- *OxyR* regulon
- Heat-shock response
- SOS regulon
  - RecA (*recA*)
- *KatF* regulon (sigma factor)
  - Exonuclease III (*xthA*)
- SoxRS regulon
  - Endonuclease IV (*nfo*)


e⁻
e⁻
e⁻
e⁻
O2
O2⁻•
H2O2
•OH
H2O

Some reactions of oxygen radicals

E. S. Henle, Y. Luo & S. Linn (1996) *Biochem.*: **35,** 12212-12219

$$O_2\bullet^- + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$

$$2\ O_2\bullet^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

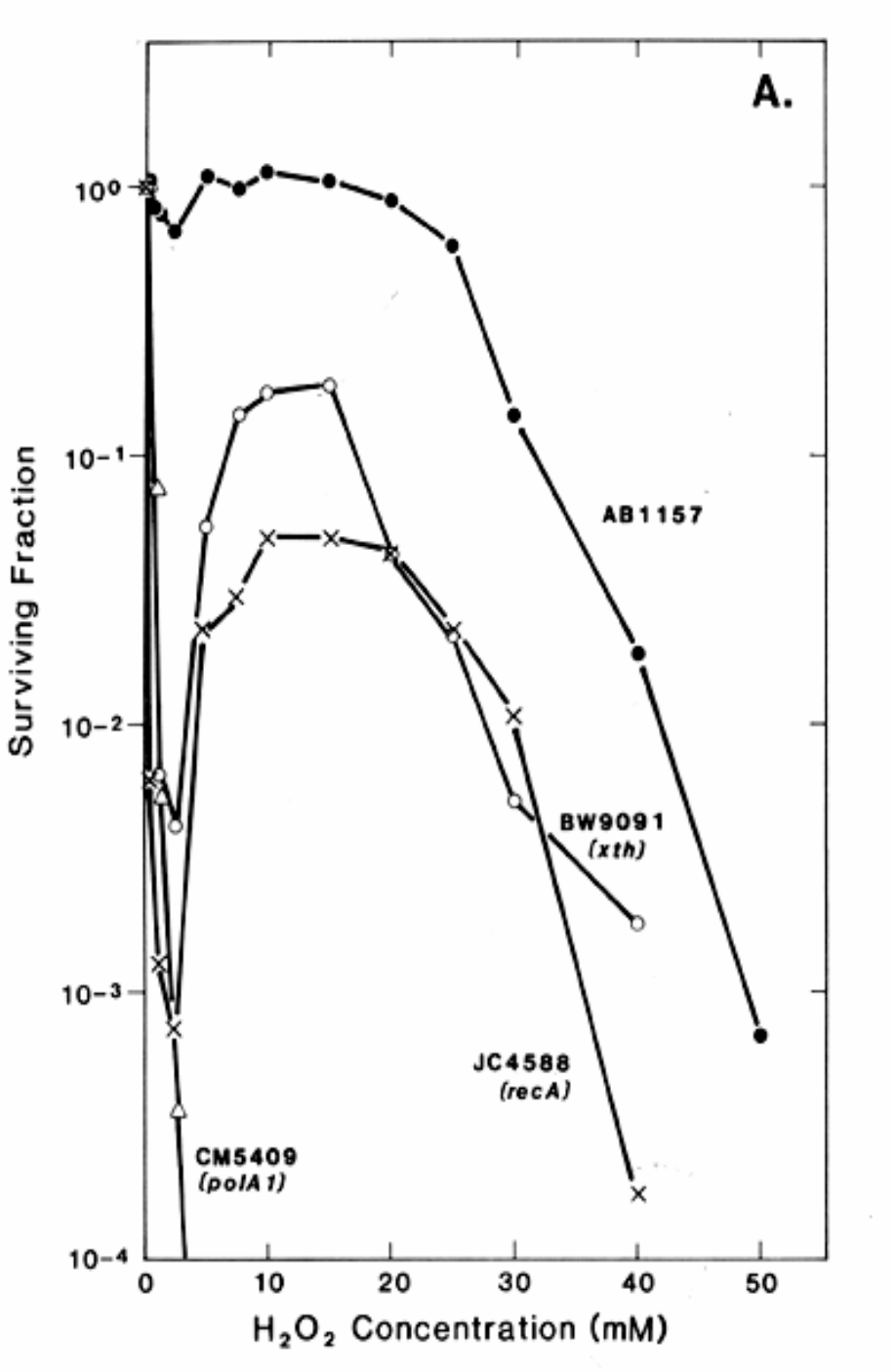
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + \bullet OH$$

$$\bullet OH + DNA \longrightarrow DNA\bullet$$

$$DNA\bullet + O_2 \longrightarrow DNAO_2\bullet$$

J. A. Imlay & S. Linn (1986)
J. Bact. **166:** 519-527
& (1987)
Ibid. **169:** 2967-2976

(1988) *Science* **240:**
640-642 & 1302-1309



Sequences of preferred duplex DNA cleavages by Fe^{2+}/H_2O_2

Type I Oxidants (0.5 mM H_2O_2)

R<u>T</u>GR

Type II Oxidants (50 mM H_2O_2)

<u>RGG</u>G, <u>TGG</u>G (?)

<u>Bold, underscored</u> nucleotides are cleavage sites "R", Purine; "Y", Pyrimidine

E. S. Henle et al. (1999) *J Biol. Chem.* **274:** 962-971

Conclusions of RTGR studies

Fe^{2+} preferentially binds to RTGR sequences because of their unique structure. That structure is not grossly perturbed upon binding.

Fe^{2+} is relatively loosely bound to RTGR; it is subject to oxidation by H_2O_2 in the unbound state, giving rise to radicals which can be quenched by H_2O_2*, thus explaining a peculiar dose response for "Mode I" damage.

$$^{*}H_2O_2 + \bullet OH \longrightarrow HO_2\bullet + H_2O$$

Promoters of genes regulating Iron Metabolism, Responses to Oxygen Radical Stress or DNA Repair genes contain RTGR in essential motifs, usually as direct or inverted repeats. Is binding at RTGR sites in these promoter regions by Fe^{2+} but not by Fe^{3+} exploited for sensing iron and/or oxygen stress and consequently regulating these genes?

P. Rai et al. (2001) *J. Mol. Biol.* **312:** 1089-1101

Conclusions of (R/T)GGG studies

Guanine N^7 is the strongest DNA coordination site for transition metals.

Breaks occur 5' to a deoxyguanosine with a 5' --> 3' polarity.

Binding of Fe^{2+} follows the same polarity.

Binding of Fe^{2+} at these sites causes a slight structural kink, somewhat stabilizing the binding and thus giving rise to the zero order dose response.

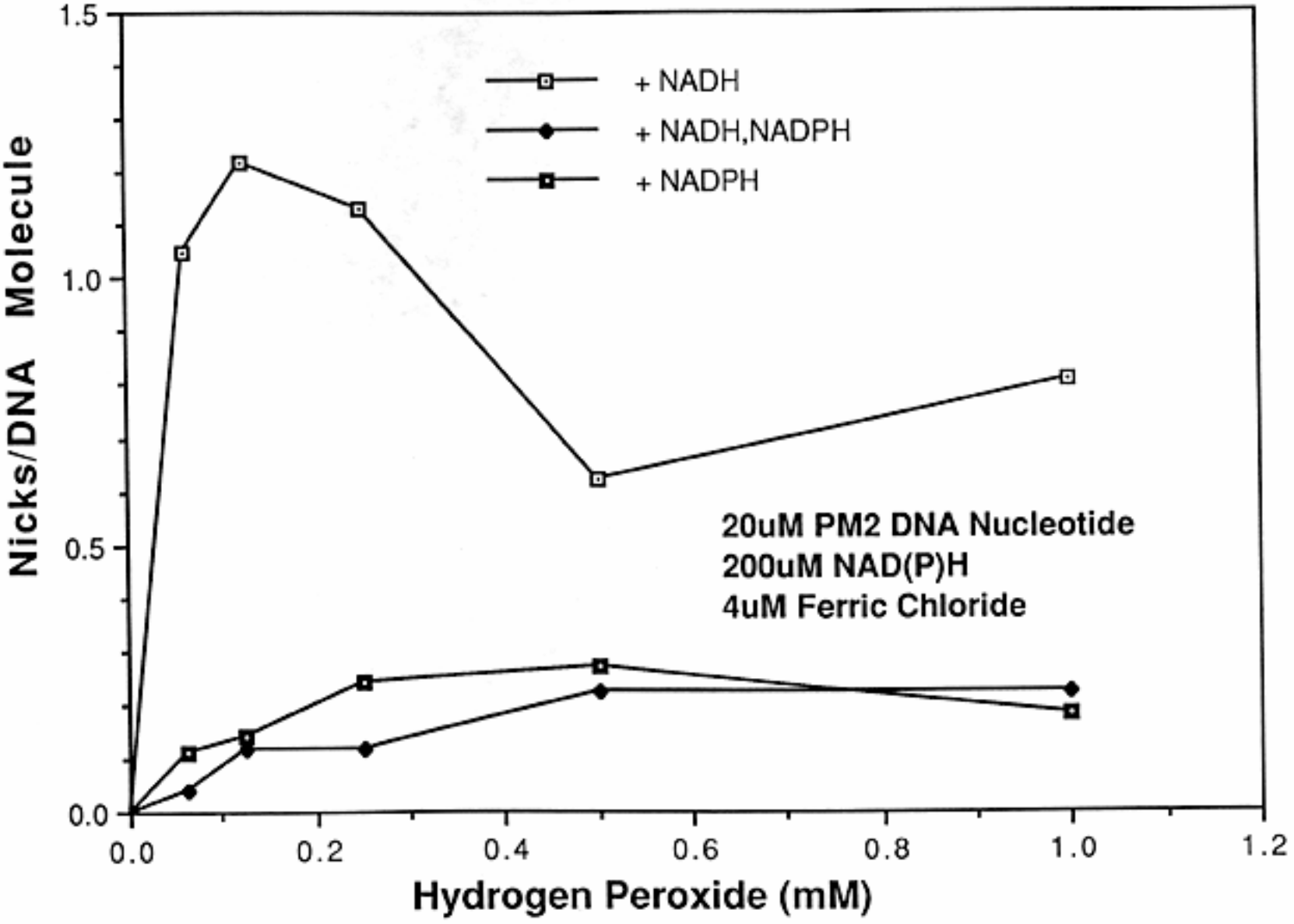
RGGG is contained in the majority of telomer repeats and telomeric sequences are cleaved preferentially *in vitro*.

Is age-related telomere shortening contributed to by iron-mediated Fenton reactions?

Human genome recombination hotspots are CCTCCCT & CCCCACCCC.

P. Rai, D. E. Wemmer, & S. Linn (2005) *Nuc. Ac. Res.* **33:** 497-510

DNA Nicking with Fe III/NAD(P)H/ Hydrogen Peroxide



NAD(P)H pools in *E. coli* 15 min after H_2O_2 challenge

H_2O_2 Challenge	Nucleotide Concentration (μM) NADH	NAD^+	NADPH	$NADP^+$	Ratio NADPH/NADH
0.0 mM	700	230	24	210	0.03
0.5	200	630	33	270	0.17
5.0	4	770	11	240	2.75
10.0	<0.1	850	8	200	>80

J. L. Brumaghim, et al. (2003) *J. Biol. Chem.* **278:** 42495-42504

Pyridine nucleotide redox state and DNA damage and repair

E. coli

NADH converted to NAD^+; NAD(P) pools ≈maintained

Mammalian Cells

Nuclear NAD^+ converted to poly(ADP)ribose and nicotinamide

Human nuclear APE1 fully inhibited by 1mM NAD^+ or ADP-ribose (I_{50} ca 40 μM), but not at all by 1mM NADH.

(The mitochondrial form is not inhibited.)

Yeast

Sir2p requires NAD^+ for deacetylase activity and the Sir proteins are involved in NHEJ and bind to the Ku complex.

Human Damage-specific DNA Binding Protein (DDB)

	DDB1	DDB2 (XPE)
Mr	127 kDa (sequence) 124,000	48 kDa (sequence) 41,000
pI	4.9	10.4
Gene map	11q12-q13	11p11-p12
Sequnce homologues	Ubiquitous (except *S. cerevisiae*)	only in mammals (CS-A is similar)

DDB binds tightly ($K_a \approx 10^{10}$) to some products of UV irradiation

T[*t*,*s*]T ≈ T[6,4]T >* T[Dewar]T > T[*c*,*s*]T* >TT

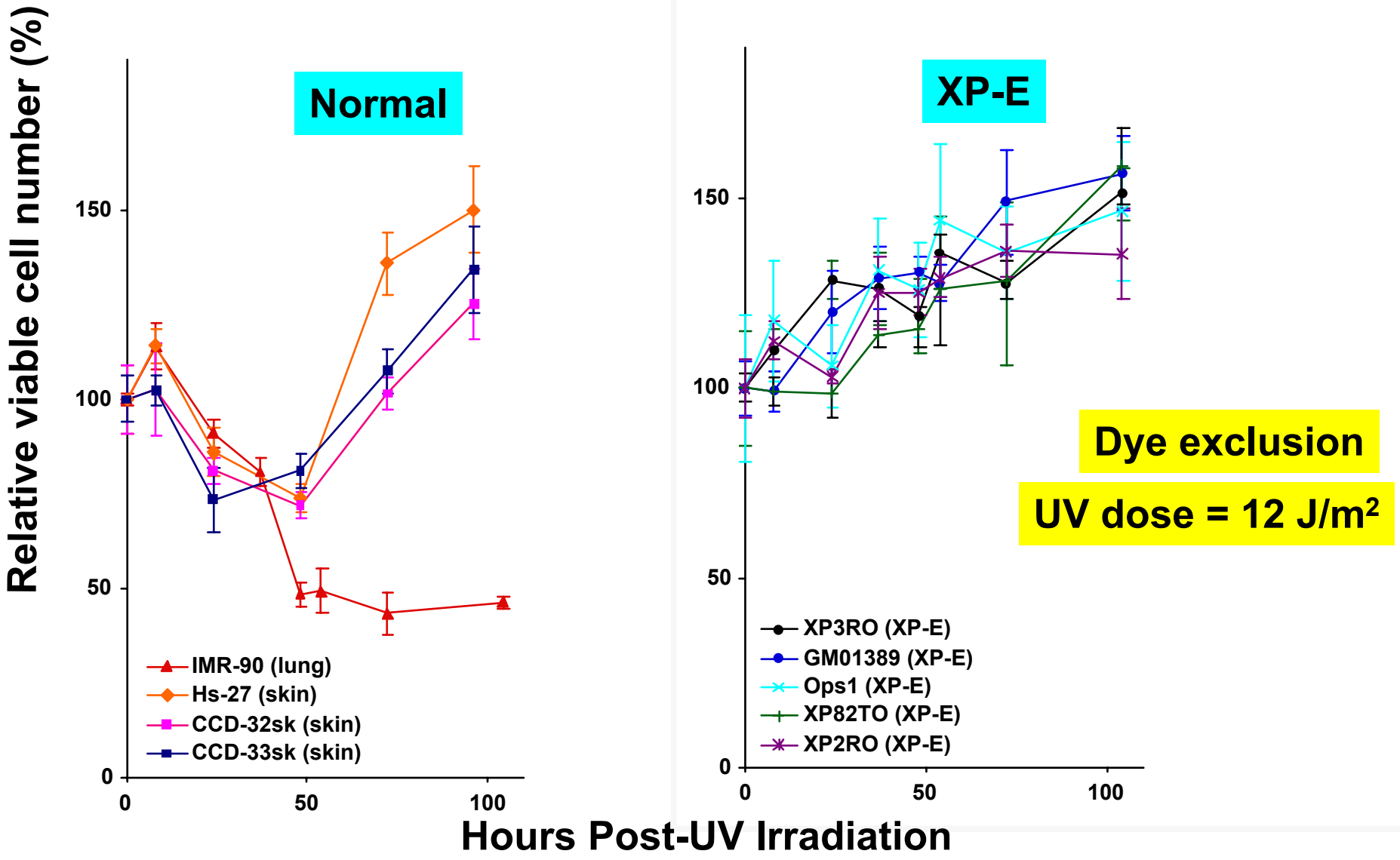
* the major UV photoproduct

* > represents a ca. 3-fold difference

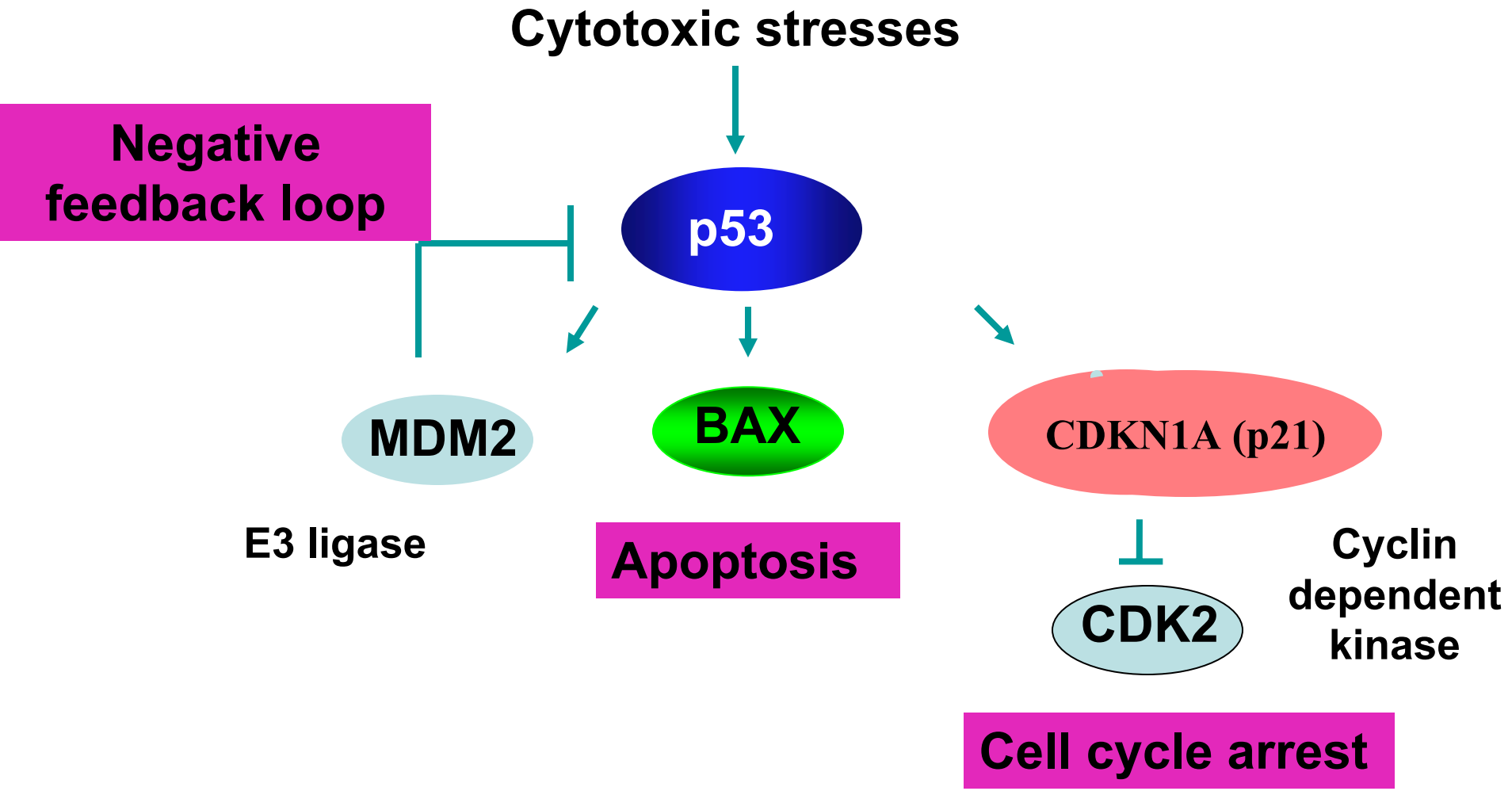
J. R. Reardon et al. (1993) *J. Biol. Chem.*: **268,** 21301-21308

10^5-10^6 copies per cell

XP-E strains are resistant to UV-induced apoptosis



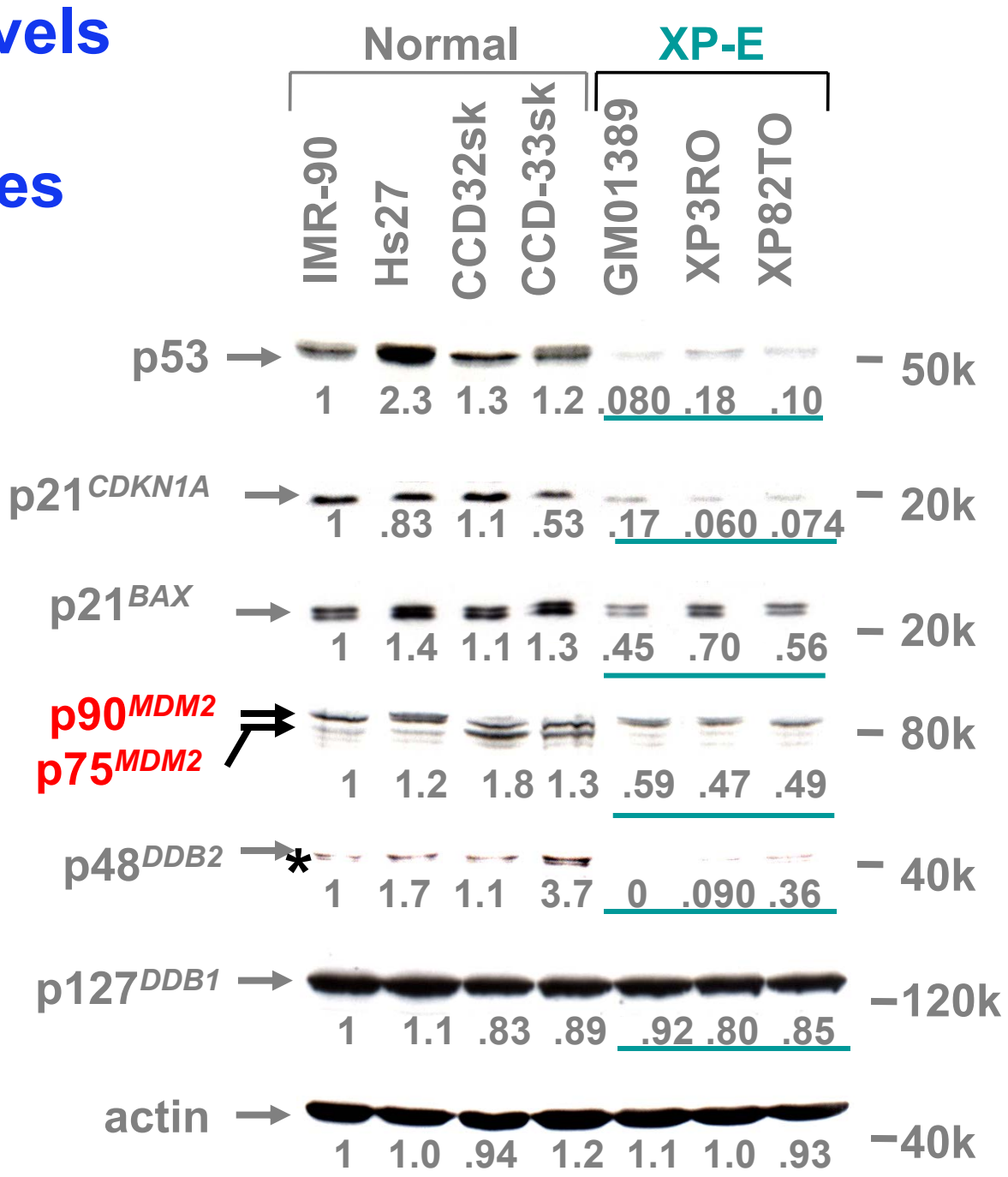
p53 regulatory pathway



No p53 mutations are found in XP-E cells.

Basal protein levels of p53 and p53-downsteam genes

Western blot



T. Itoh, C. O'Shea & S. Linn (2003) *Mol. Cell Bio.* **23:** 7540-7553

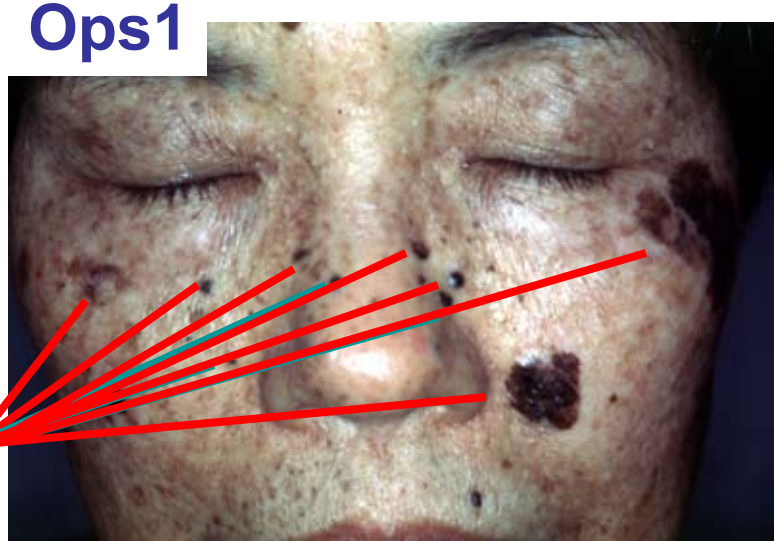
Tentative proposal for carcinogenesis in XP-E

Abnormally high DDB2 mRNA expression and/or low DDB2 protein levels → Diminished p53 levels

↓

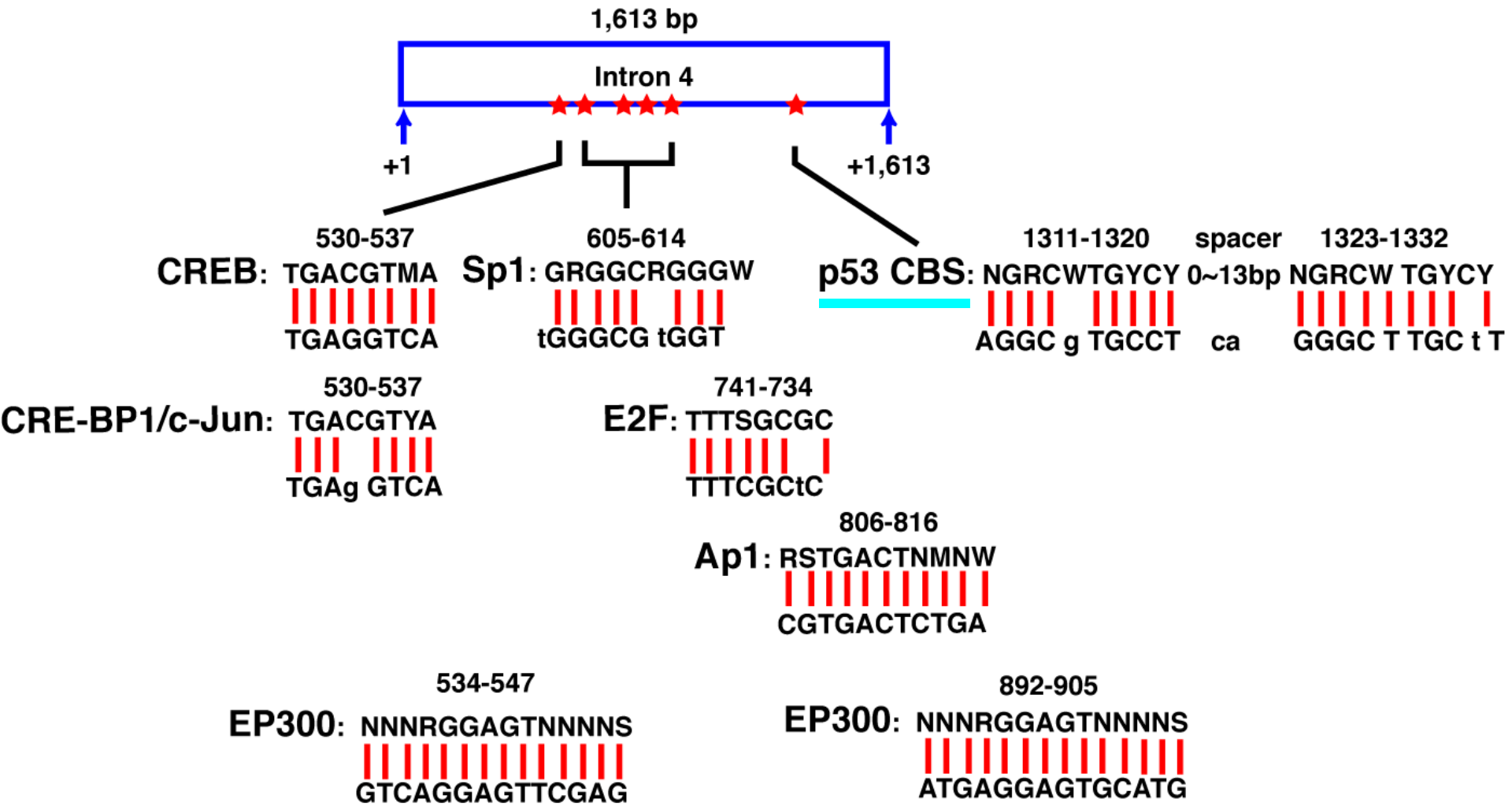
Diminished apoptosis and cell cycle arrest after UV → XP-E

Ops1

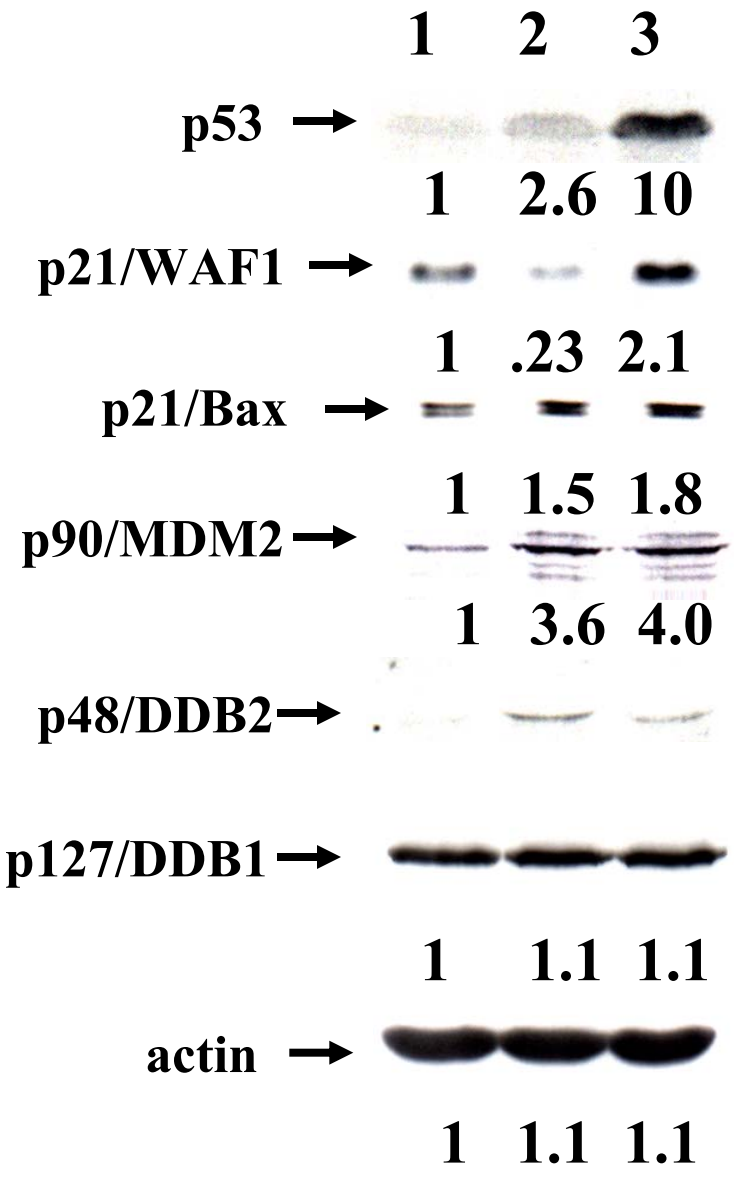


Skin carcinogenesis

Putative regulatory elements of intron 4 of the DDB2 gene



Effect of the three DDB2 expression constructs upon basal protein levels in an XP-E strain

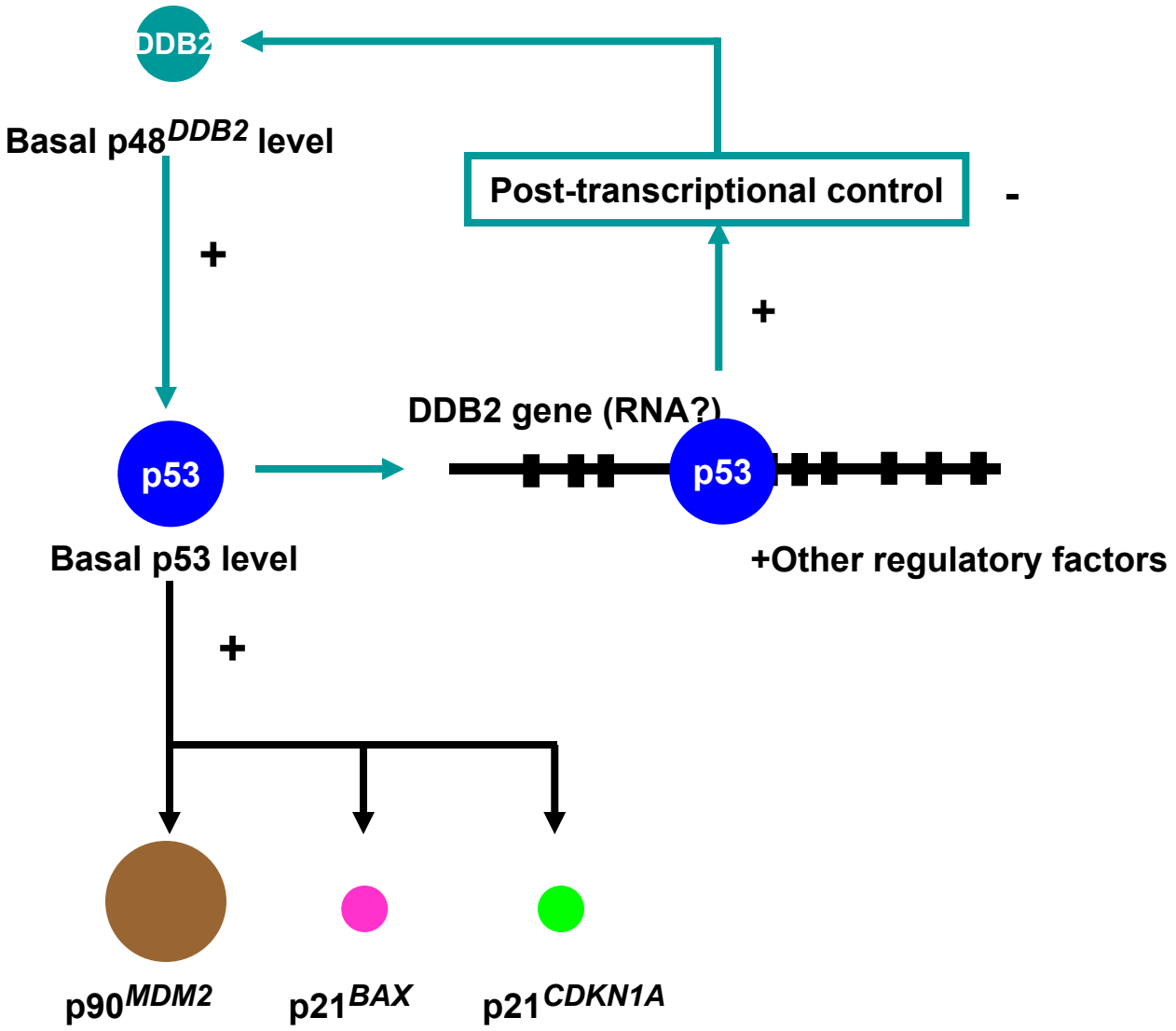


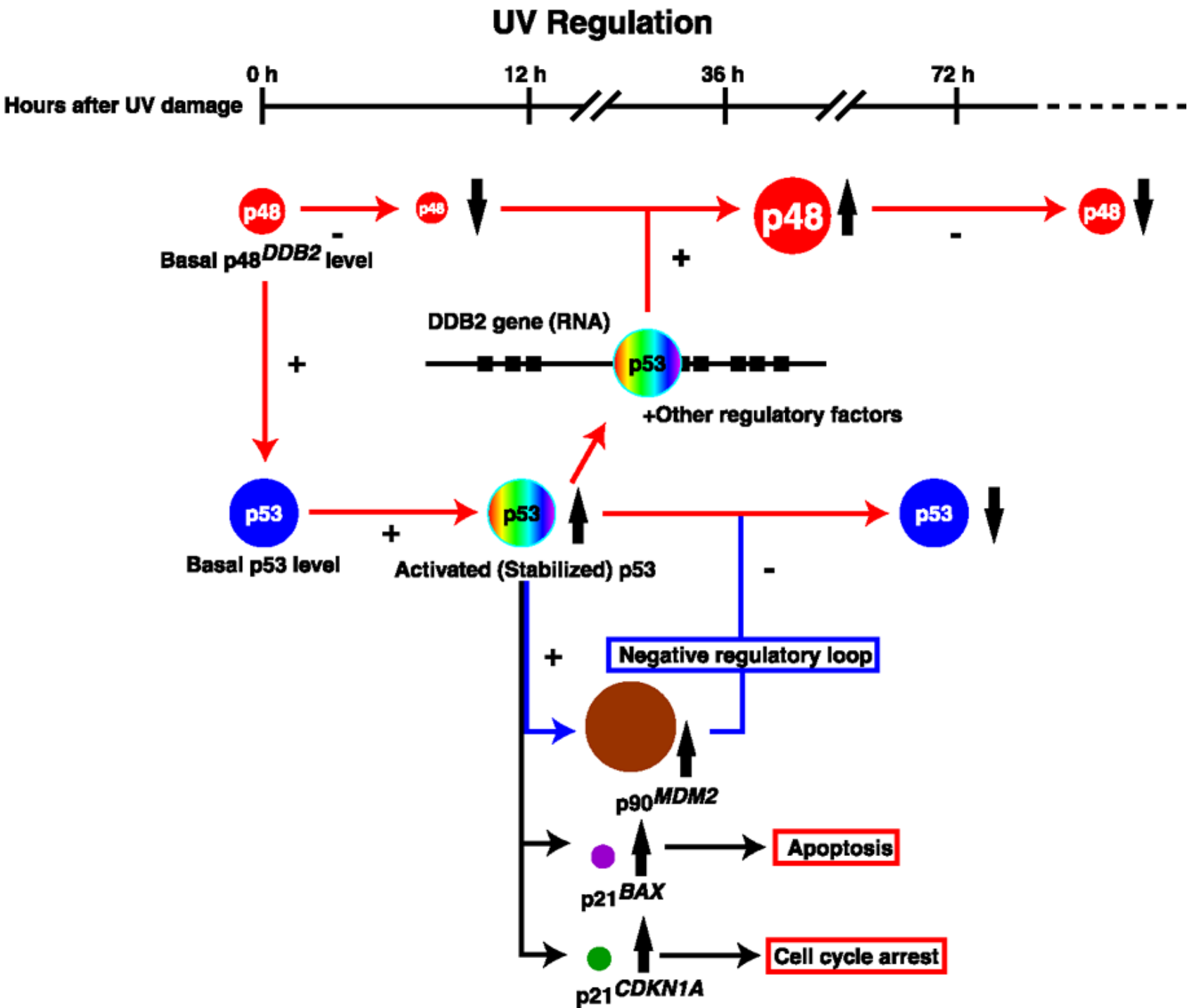
1: 5'UTR
2: 5'UTR+hDDB2cDNA
3: 5'UTR+cDNA+Intron 4

Western Blot

Regulation of basal levels

Basal DDB2 level is kept low and stable.




UV Regulation
0 h
12 h
36 h
72 h
Hours after UV damage
p48
Basal p48DDB2 level
DDB2 gene (RNA)
p53
+Other regulatory factors
Basal p53 level
Activated (Stabilized) p53
Negative regulatory loop
p90MDM2
p21BAX
Apoptosis
p21CDKN1A
Cell cycle arrest

DDB subunits bind to transcription and cell cycle regulators

Bound protein/complex	DDB1	DDB2	Reporting Laboratories
HBV protein X	X	X	Janet Butel & Pradip Raychaudhuri
EBV EBNA 2	X		Stuart Linn
Paramyxoviridae V proteins	X		Robert Lamb
E2F1		X	Pradip Raychaudhuri
CBP/p300	X	X	Pradip Raychaudhuri & Vesna Rapic-Otrin
STAGA	X	X	Robert Roeder
Cullin 4A in COP9 signalosome	X	X	Pradip Raychaudhuri & Yoshihiro Nakatani
CDT1	X		Yue Xiong

A possible scenario for DDB2 and global genomic repair*

- **After UV irradiation, DDB heterodimer binds DNA UV damages.**
- **The bound DDB recruits the STAGA and CBP/p300 protein acetylase/chromatin remodeling complexes.**
- **XPC or XPA is recruited to the site, depending on the damage.**
- **Within the first ≈60 min repair takes place and DDB recruits the Cullin 4A ubiquitin ligase off of the COP9 signalosome.**
- **DNA repair factors, DDB, and chromatin remodeling complexes are ubiquitinated by the recruited Cullin 4A and then degraded by the proteasome within ≈120 min. (CBP/p300 degradation would allow p53 to accumulate; STAGA and CBP/p300 degradation would limit chromatin remodeling.)**
- **DDB2 is restored after repair is complete (after 36 hr.)**
- **DDB1 is transported to the nucleus, E2F1 and CDT1 are bound, cell cycle progression resumes, and the DDB system is re-primed.**

*Based upon observations from the laboratories of P. Raychaudhuri, V. Rapic-Otrin, R. Roeder, Y. Nakatani, T. Matsunaga, K. Sugasawa, J. Ford, and others

Notes on the putative scheme

- **The time-ordering of early events may not be exact.**
- **It is not clear whether DDB1, DDB2, or the heterodimer binds the various complexes/proteins at various stages.**
- **If all lesions are repaired within 60 minutes, subsequent events, including those mediated by p53, do not occur.**
- **For *transcription-coupled excision repair* (TCR) a similar scenario appears to take place with CSA replacing DDB2 in some or all functions. ---Nakatani lab.**
- **If DDB2 were to function to coordinate the repair scenario with *p53-mediated checkpoint and apoptosis* responses, then the presence of DDB2 only in higher eucaryotes *versus* the ubiquitous presence of DDB1 would be explained.**

Properties of DDB2$^{-/-}$ mice

Itoh *et al.* (2004) *Proc. Natl. Acad. Sci.* USA **10:** 2052-2057

- **F2 mice are viable, fertile, 92% of normal weight (8 wks.)**
  (F6 mice are fertile, but becoming smaller.)

- **Primary fibroblasts (MEFs) lack DDB2 expression as assayed by RT-PCR, activity, and immunoblotting.)**
  (Heterozygotes' cells have ≈ 1/2 normal levels.)

- **Primary fibroblasts (MEFs) resemble human XP-E fibroblasts (hyper-resistant to UV; reduced apoptosis and expression of p53-mediated effectors). (Heterozygotes' cells are normal.)**

- **Predisposition to squamous cell carcinomas induced by UV, but not by DMBA (7,12-dimetylbenz[a]anthracene).**

Properties of DDB2$^{-/-}$ mice

Itoh *et al.* unpublished (June 2006)

- **UV-B caused cataracts only in -/- mice when mice treated with 2,500J/m^2 for 5 days per week for up to 20 weeks.**
- **Life spans of -/-, -/+, +/+ mice not statistically different.**
- **-/- mice not abnormally sensitive to 400 rads of γ-radiation given at 7-1/2 to 8-1/2 weeks.**
- **E2F1 and p53 -/- phenotypes appear to be unaffected by DDB2 genotype.**

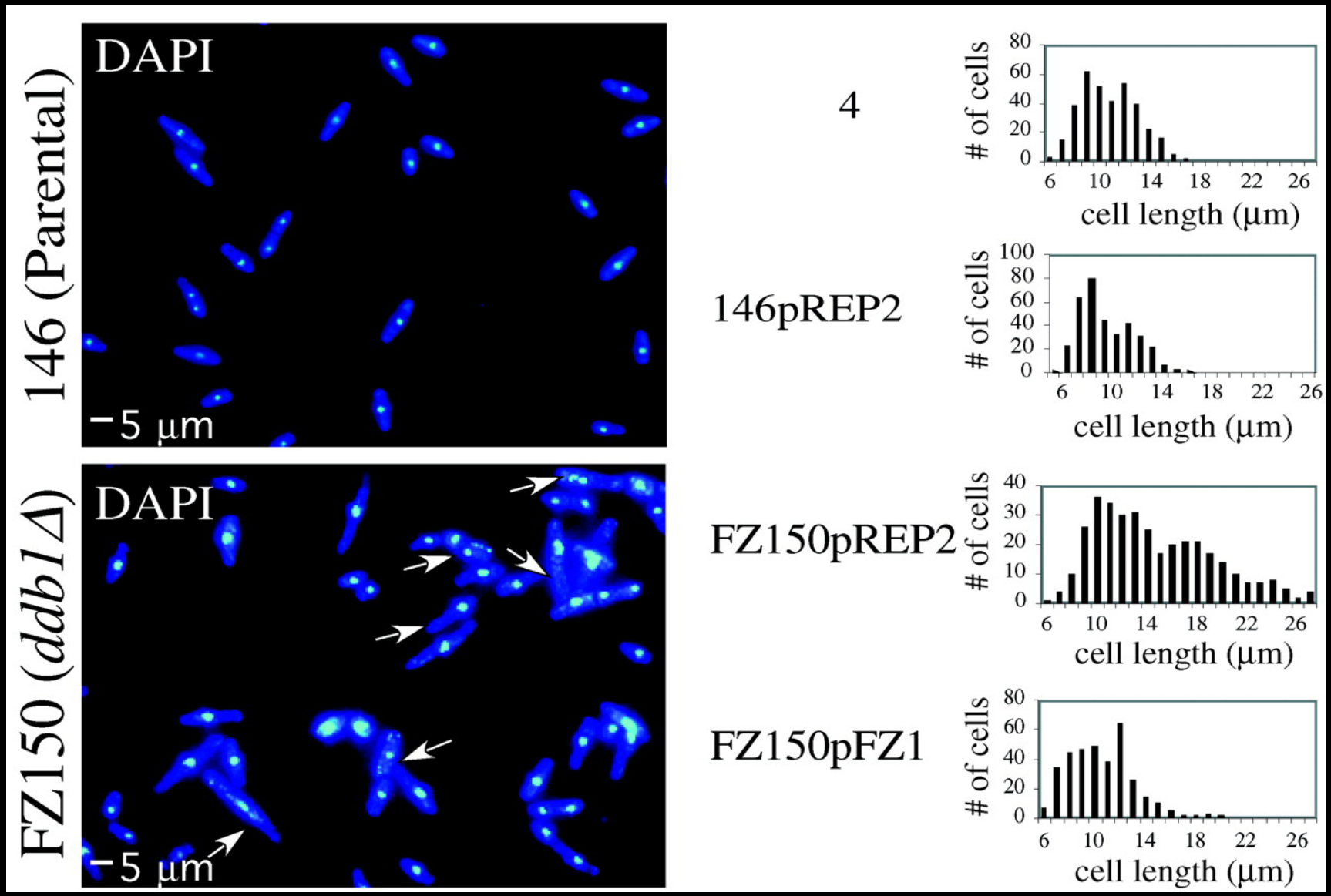
For the future….

- **Molecular details of the regulatory interactions between DDB2 and p53?**

- **Is DDB2 involved in signaling events in response to genotoxic stresses other than UV?** **(Possibly not, but cisplatin induces DDB2 roughly 4.5-fold.)**

- **Are there physiological functions of DDB1 in mammals in the absence of DDB2?**

  [DDB1 (conditional?) KO mice?]



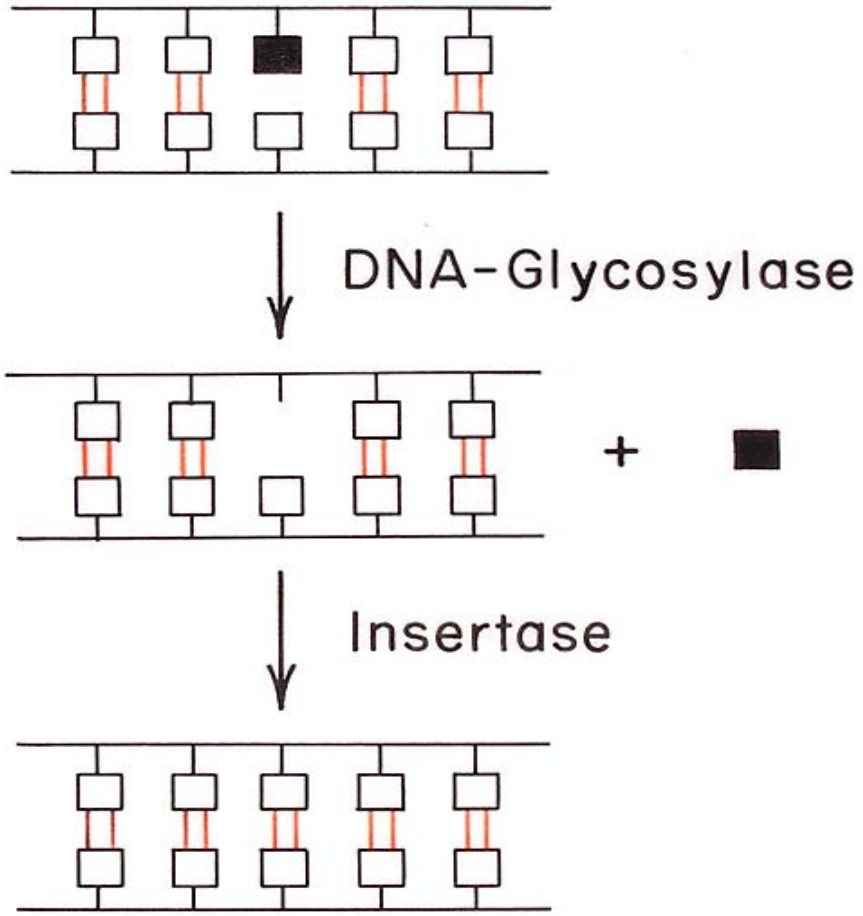
F. Zolezzi, et al. (2002) *J. Biol. Chem.* **277:** 41183-41191

On the roles of pol ε and DDB1

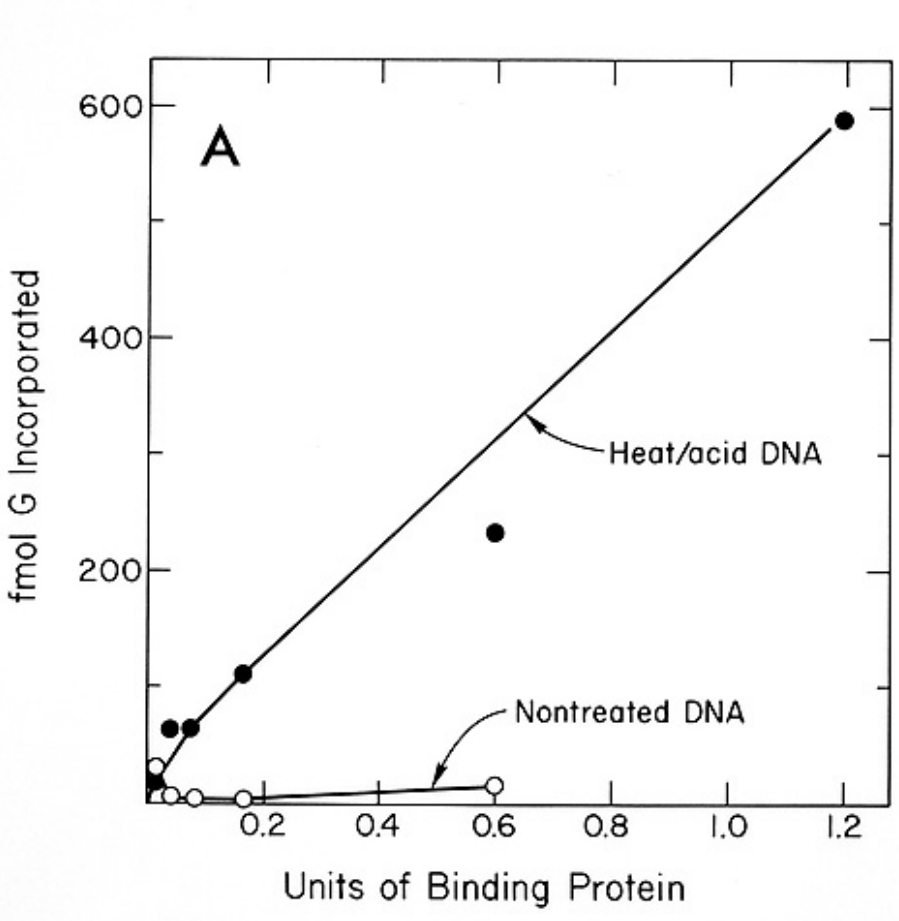
- Human pol ε and DDB co-purify and human DDB1 coIP's with the pol ε catalytic subunit, p261.
- DDB1 and pol ε have both been associated with heterochromatin structure maintenance.
- Do pol ε and DDB1 act jointly for regulating S phase progression in mammals and perhaps other organisms?
- Does pol ε regulate S phase progression alone only in *S. cerevisiae* and other organisms that lack DDB1?

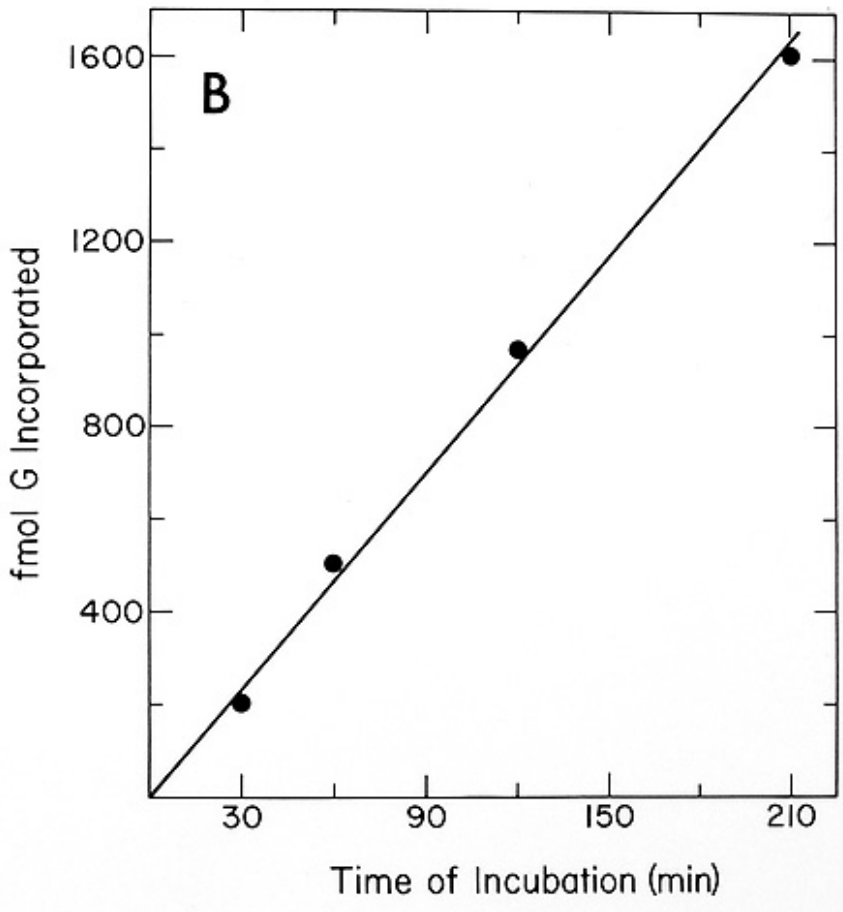
  (Has "the awesome power of yeast genetics" misguided us?)
- Or, does DDB1 alone regulate S phase progression in mammals and perhaps other organisms?
- But then, what of the interaction of DDB1and pol ε in humans?

DNA REPAIR VIA BASE INSERTION



W. A. Deutsch & S. Linn (1979) *PNAS* **76**: 141-144 & *JBC* **254**: 12099-12103


A
600
400
200
fmol G Incorporated
Heat/acid DNA
Nontreated DNA
0.2
0.4
0.6
0.8
1.0
1.2
Units of Binding Protein


B
1600
1200
800
400
fmol G Incorporated
30
90
150
210
Time of Incubation (min)

Properties of Insertase

- Specific apurinic (heat/acid treated) DNA Binding
  - Does not bind to nicked apurinic sites (which inhibit)
  - G and A remove protein from bound sites
  - Activity heat- and cold-labile
- Insertion
  - G and A, but *not* T, C, dN, dNTP or NTP are substrates
  - Insertion makes sites stable to alkali and AP endonuclease
  - K_m 5μM for G; ≤5μM for A; inserts 40-400 purines/hr.
  - Product of G incorporation recovered as dGMP after hydrolysis of the DNA with DNase and SVD.
  - Activity heat- and cold labile
  - Requires K^+

W. A. Deutsch & S. Linn (1979) *PNAS* **76**: 141-144 & *JBC* **254**: 12099-12103

“There are two types of scientists in the world: turbidifiers and clarifiers.”

---Sydney Brenner

Acknowledgements

- My mentors
- “We” --some 150 undergraduate & graduate students, career researchers, postdocs, and visitors in our lab at Berkeley
- Collaborators worldwide
- Colleagues (including competitors) worldwide

This talk is dedicated to
Alex Karu (1943-2006)
Dale Mosbaugh (1953-2004)