“History of DNA Repair”

Samuel H. Wilson
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Transforming technologies along the way: A story about Pol ß & BER

1970s: Column chromat. (DEAE & PC); peptide mapping; activity gel assay; IPs and immunoaffinity purifications

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Case in point: A novel co-factor in mammalian BER (HMGB1)

i) Identification by interaction with BER intermediate and MS
ii) Recruitment at sites of DNA damage in a living cell

Looking for cellular RT…

Reprinted from
Proc. Nat. Acad. Sci. USA
Vol. 69, No. 6, pp. 1531–1536, June 1972

(endogeneous retroviral particle in some mouse tumors)

A Novel DNA Polymerase Activity Found in Association with Intracisternal A-Type Particles

(DNA synthesis/tumors/virus-like particles/ poly(rA)/myeloma)

SAMUEL H. WILSON AND EDWARD L. KUFF

Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Communicated by Marshall Nirenberg, April 11, 1972

ABSTRACT **A DNA polymerase activity that promotes the synthesis of poly(dT) has been found in association with intracisternal A-type particles isolated from several mouse tumors. The poly(dT) synthesis activity requires a DNA or RNA primer, is optimal at high salt concentration, prefers magnesium over manganese, and is stimulated by poly(rA). No significant incorporation of dAMP, dGMP, or dCMP was detected in the presence of several RNA and DNA template–primers. The enzyme activity differs in several of its properties from the poly(rA)-directed DNA polymerase activity associated with Rauscher murine leukemia virus.**

A reverse transcriptase with unique enzymatic specificity compared with other RTs, Pol I & the known cellular polymerase: Template use; KCl resistance; metal ion specificity.

Hence, DNA polymerases have unique fingerprints with regard to catalytic specificity.

Strong DNA polymerase activity…

incubation time

protein

dNTP

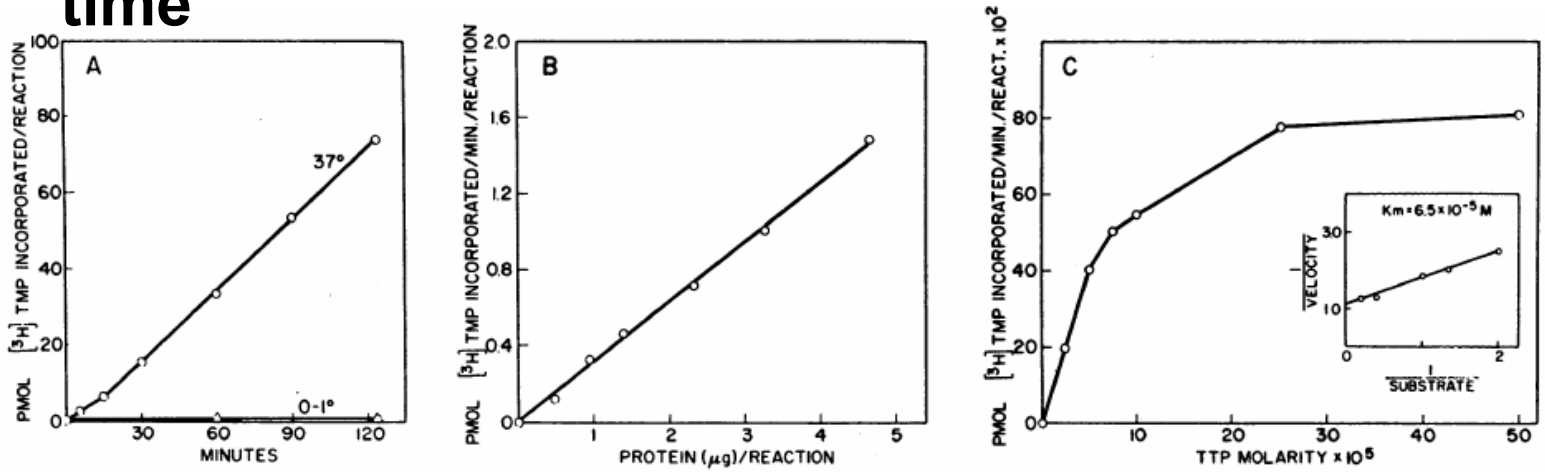


FIG. 1. The relation between [³H]dTMP incorporation and time of incubation (*A*), protein concentration (*B*), and [³H]dTTP concentration (*C*). dTTP was the only deoxynucleotide present.

Catalytic specificity of DNA polymerases

TABLE 4. *Response of DNA polymerase activities in A-type particles and MuLV to various template–primers*

Modification	A-type particle conditions	MuLV conditions			
		A-type particles†		MuLV†	
	A-type particles*	12.5 mM Mg^{++}	1.5 mM Mn^{++}	12.5 mM Mg^{++}	1.5 mM Mn^{++}
	pmol [^3H]dTMP incorp./min per mg protein				
Minus template–primer	0.1	0.2	0	0	21
Poly(rA)·$(dT)_{14}$	296	64	13	302	4910
Poly(rA)·$(dT)_6$	342	—	—	—	—
Poly(rA)·(dT)	86	—	—	—	—
Poly(rA)·(rU)	3	—	—	—	—
Poly(rA)·$(dG)_{14}$	0	—	—	—	—
Poly(rA)	0	—	—	—	—
Poly(rU)	0	—	—	—	—
Poly(rC)·$(dG)_{14}$	0	—	—	—	—
"Activated" calf-thymus DNA	0	0.5	0.7	80	16
Native calf-thymus DNA	0.4	—	—	—	—
Denatured calf-thymus DNA	0	—	—	—	—
Poly d(A-T)	0	0.5	1.4	770	246
Poly(dC)·(dG)	0	—	—	—	—
Poly(dA)·$(dT)_{14}$	0	—	—	—	—
Poly(dA)	0	—	—	—	—

Reprinted from
Proc. Nat. Acad. Sci. USA
Vol. 71, No. 2, pp. 578–582, February 1974

Looking for a Cellular RT…

1974

Multiple Forms of DNA Polymerase in Mouse Myeloma

(systematic fractionation/poly(rA)·oligo(dT) template-primer/tumors)

A. MATSUKAGE, E. W. BOHN, AND S. H. WILSON

Laboratory of Biochemistry, National Cancer Institute National Institutes of Health, Bethesda, Maryland 20014

Communicated by Robert W. Berliner, September 24, 1973

ABSTRACT **Five distinct forms of DNA polymerase (deoxynucleosidetriphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7) were separated from extracts of mouse myeloma MOPC-104E using a fractionation procedure based upon sequential ion-exchange column chromatography. The enzymes were characterized according to sedimentation behavior, subcellular localization, chromatographic behavior on hydroxyapatite columns, and reaction properties. The results indicate that myeloma contains two enzymes that appear to correspond to well characterized DNA polymerases found in many other mammalian tissues, a 6S DNA polymerase localized in the cytoplasmic supernatant fraction, and a lower molecular weight (2–3S) DNA polymerase. Also present were a second 6S DNA polymerase localized exclusively in the nuclear fraction and a 6–8S DNA polymerase localized in the cytoplasmic membrane fraction. The enzyme in the cytoplasmic membrane fraction, which accounted for the predominant activity in the myeloma, was active with poly(rA)·$(dT)_{12-18}$ as template-primer, but not with activated calf thymus DNA. The detection of this distinct 6–8S membrane-bound DNA polymerase is of particular interest.**

Fractionation of crude extract: Assays specific for RT and Pols alpha & beta.

Pol alpha

Pol beta

Pol delta

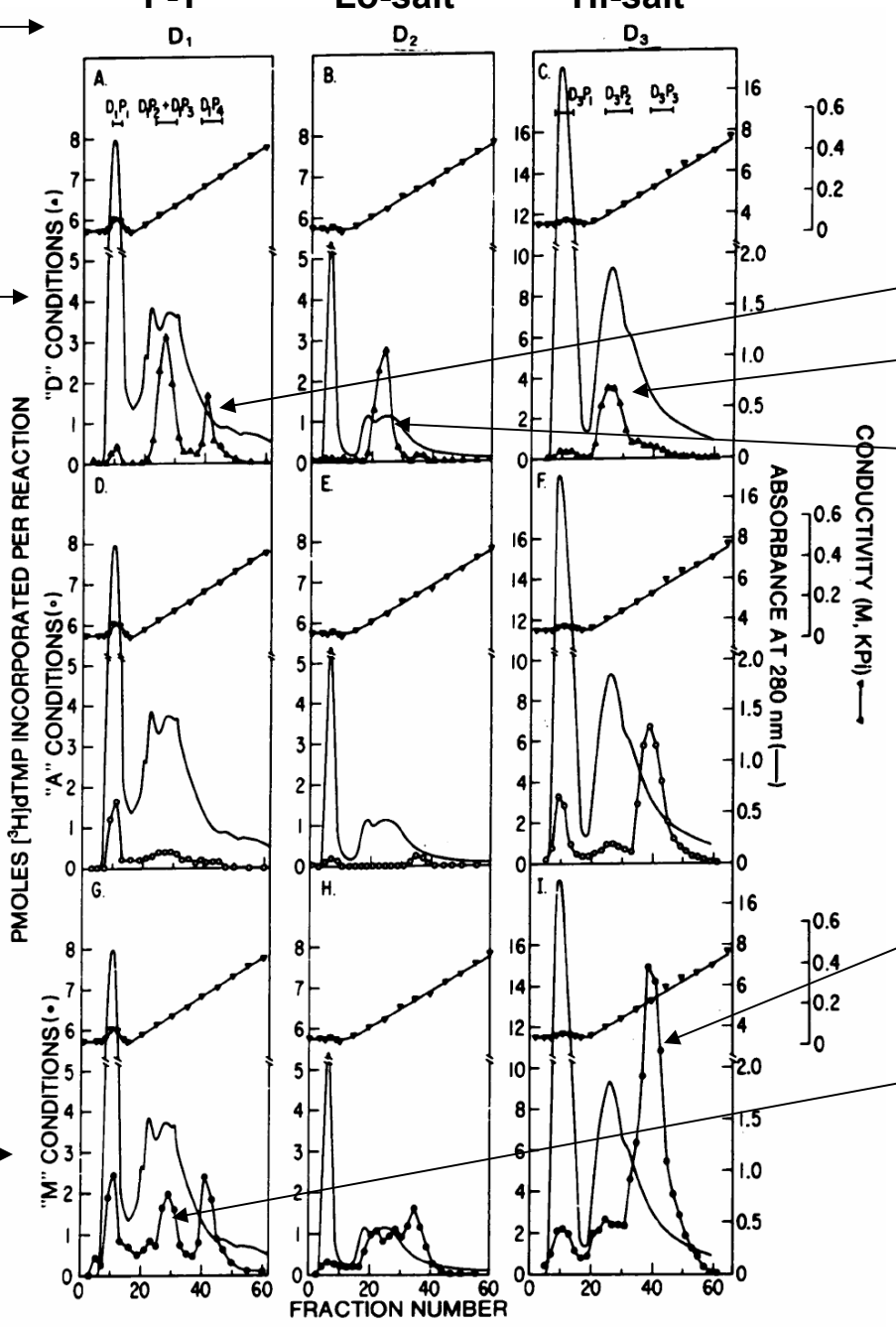
Pol gamma

3 DEAE-cellulose fractions

F-T

Lo-salt

Hi-salt



Activated DNA as T-P

Poly (rA)-oligo (dT) as T-P

Pol ß

Pol α_1

Pol α_2

Pol γ

Endogenous MuLV-like Retroviral RT

3 Phosphocellulose columns

[Reprinted from Biochemistry, (1975) 14, 1006.]

1975

On the DNA Polymerase III of Mouse Myeloma: Partial Purification and Characterization†

A. Matsukage, E. W. Bohn, and S. H. Wilson*

Pol γ

ABSTRACT: A high molecular weight membrane-bound DNA polymerase from the mouse myeloma, MOPC-104E, has been purified extensively, and characterized with regard to physical and reaction properties. This enzyme, which is readily distinguishable from other myeloma enzymes that are analogous to the recognized forms of cellular DNA polymerase, is designated DNA polymerase III. DNA polymerase III activity in whole homogenates from MOPC-104E was solubilized and then purified using a series of ion-exchange chromatographic procedures followed by DNA-cellulose chromatography and glycerol gradient centrifugation; the enzyme activity, as measured with poly(rA) · $(dT)_{12-18}$ as template–primer and Mn^{2+} as divalent cation, was purified as much as 18,000-fold. In the final stages of the purification, DNA polymerase III possessed no detectable RNA polymerase activity, nucleoside diphosphokinase activity, or nuclease activity toward DNA or single- and double-stranded RNA. Ribonuclease H activity was present through most of the purification. However, this activity did not precisely copurify with the DNA polymerase activity, and was completely separated from DNA polymerase III in the final step of the purification. The isoelectric point of the purified DNA polymerase III was approximately pH 5.8; the approximate molecular weight under nondissociating conditions was 315,000 by gel filtration, 270,000 by gel electrophoresis, and 230,000 by sedimentation velocity (relative to catalase at 243,000). As revealed by glycerol gradient centrifugation, the enzyme was not dissociated into smaller species possessing activity by treatment with either 125 mM ammonium sulfate, RNase and DNase, or 500 mM KCl and 0.2% Tween-80. In solutions of relatively low ionic strength and under certain other conditions, the enzyme aggregated into a much higher molecular weight species. Activity of DNA polymerase III was inhibited by sulfhydryl-blocking reagents and by 1,10-phenanthroline; it was not inhibited by antiserum directed against MuLV DNA polymerase. DNA polymerase III required a base pair complementary combination of template, primer, and deoxynucleoside 5′-triphosphate for activity; as template it preferred poly(rA), poly(dA), and poly(dC), and was relatively inactive with calf thymus DNA, poly(rI), poly(rC), and poly(dG) under the conditions tested. The template specificity varied depending upon whether Mn^{2+} or Mg^{2+} was the divalent cation. Several properties of the polymerase activity such as pH optima, activation energy, etc., were different in reactions with poly(rA) · $(dT)_{12-18}$ and Mn^{2+} as divalent cation than in reactions with poly(dA) · $(dT)_{12-18}$ and Mg^{2+} as divalent cation. This suggests that the precise nature of the enzyme reaction under the two conditions is not identical, yet the active sites appear to reside in the same protein complex. Adult BALB/c mouse liver was found to contain a DNA polymerase with chromatographic and sedimentation properties virtually identical with those of the MOPC-104E DNA polymerase III. This liver enzyme was not detected in either purified mitochondria or in the cytoplasmic S100 fraction. Thus, an enzyme that appears analogous to the myeloma DNA polymerase III is present in a normal mouse tissue.

1979

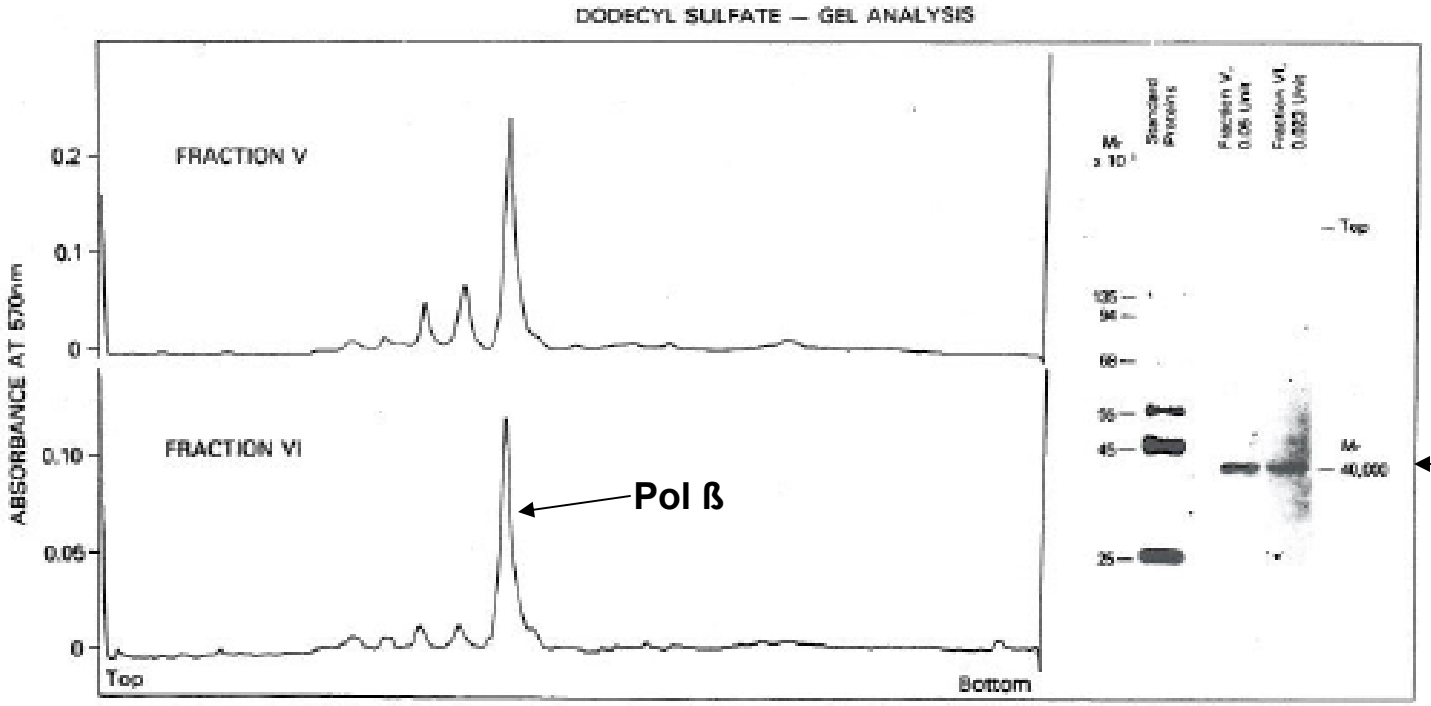
Published in **BIOCHEMISTRY**, July 1979, pp. 3401–3406, by the American Chemical Society

Steady-State Kinetics of Mouse DNA Polymerase β[†]

K. Tanabe, E. W. Bohn, and S. H. Wilson*

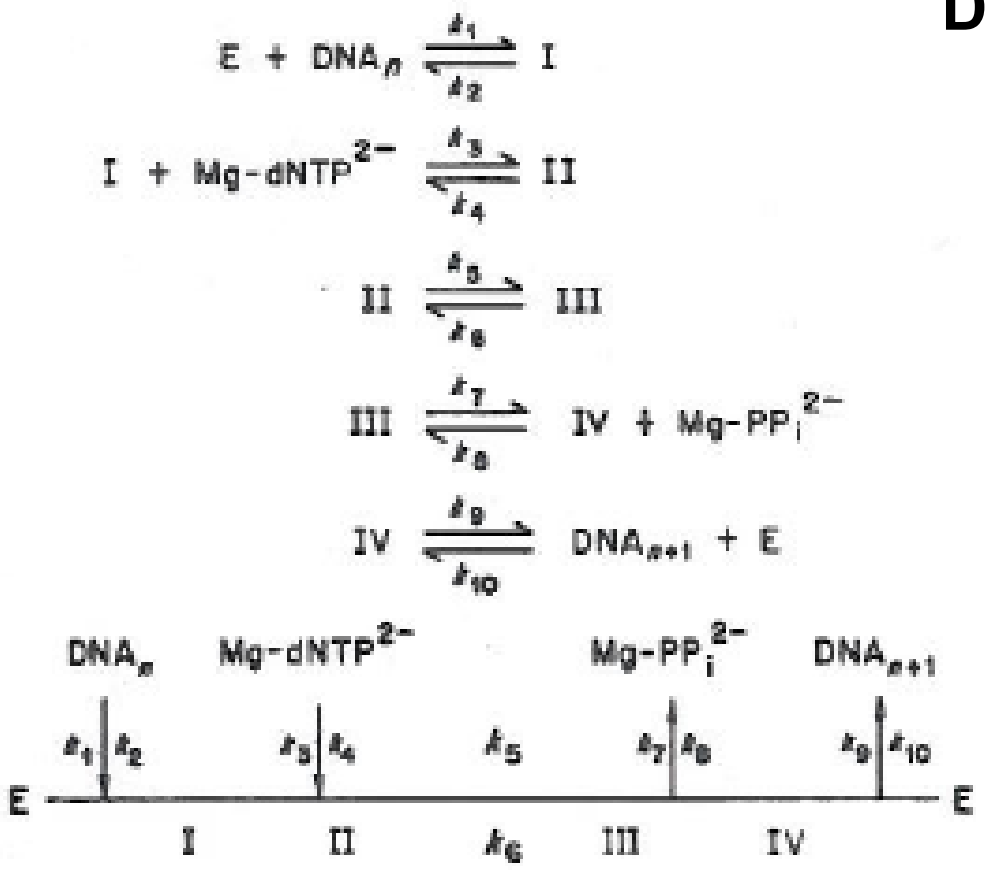
ABSTRACT: DNA polymerase β from mouse myeloma has been purified to near homogeneity, and its properties have been examined. The enzyme did not catalyze a detectable level of dNTP turnover, pyrophosphate exchange, pyrophosphorolysis, 3′-exonuclease degradation, or 5′-exonuclease degradation. Steady-state kinetic studies point to an ordered bibi mechanism for the polymerization reaction. Metal activation, which is required for polymerization, did not alter the K_m for either the dNTP or the template–primer.

purification and kinetics



Yield in Fraction VI: ~5 µg/200 g tissue or batch (!)

DNA adds first…



Ordered sequential mechanism

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 254, No. 22, Issue of November 25, pp. 11678-11687, 1979
Printed in U.S.A.

1979

Mouse DNA Polymerase α

SUBUNIT STRUCTURE AND IDENTIFICATION OF A SPECIES WITH ASSOCIATED EXONUCLEASE*

(Received for publication, April 27, 1979)

Yang-Chang Chen, Elizabeth W. Bohn, Stephen R. Planck,‡ and Samuel H. Wilson

From the Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Two species of α-polymerase with very similar catalytic properties have been purified to near homogeneity from a soluble protein fraction of mouse myeloma. Sedimentation analysis in 0.5 M salt-containing glycerol gradients indicated that both species had a native M_r of about 190,000. Each species contained nonidentical subunits with apparent molecular weights of about 47,000 and 54,000. Subunits of M_r = ~50,000 had been found previously in calf thymus α-polymerase (Holmes, A. M., Hesslewood, I. P., and Johnston, I. R. (1974) *Eur. J. Biochem.* 43, 487-499; (1976) *Eur. J. Biochem.* 62, 229-235). Tryptic peptide mapping failed to reveal primary structure homology between the subunits of the two enzymes. Thus, the two α-polymerases are clearly different species. These two enzymes are further distinguished by the fact that one of them has asssociated exonuclease activities. One activity degraded single-stranded DNA to mononucleotides in the 3′ → 5′ direction and acted distributively. The other exonuclease activity also degraded single-stranded DNA to mononucleotides, but this degradation was in the 5′ → 3′ direction in a processive fashion. Both exonuclease activities co-migrated with the polymerase activity during the final purification step of polyacrylamide gradient gel electrophoresis, which yielded the essentially homogenous α-polymerase, and also during sedimentation of the purified enzyme through a high salt glycerol gradient.

Two Pol α species: Native M_r in range of 190,000, both with subunits in the range of 50,000.

Questions: Is Pol ß a proteolytic fragment of Pol α ?

What is the M_r of the catalytic subunit of Pol α ?

Transforming technologies along the way: A story about Pol ß & BER

1970s: Column chromat. (DEAE & PC); peptide mapping; activity gel assay; IPs and immunoaffinity purifications

1980s: cDNA cloning; expression in *E. coli*; FPLC at room temperature

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2000—: IPs & blotting; crystallography with non-hydrolyzable dNTPs; sub-nuclear imaging; affinity capture & mass spectrometery

Case in point: A novel co-factor in mammalian BER (HMGB1)

i) Identification by interaction with BER intermediate and MS
ii) Recruitment at sites of DNA damage in a living cell

Volume 8 Number 12 1980 1980 Nucleic Acids Research

Distinction between mouse DNA polymerases α and β by tryptic peptide mapping

S.R.Planck, K.Tanabe and S.H.Wilson

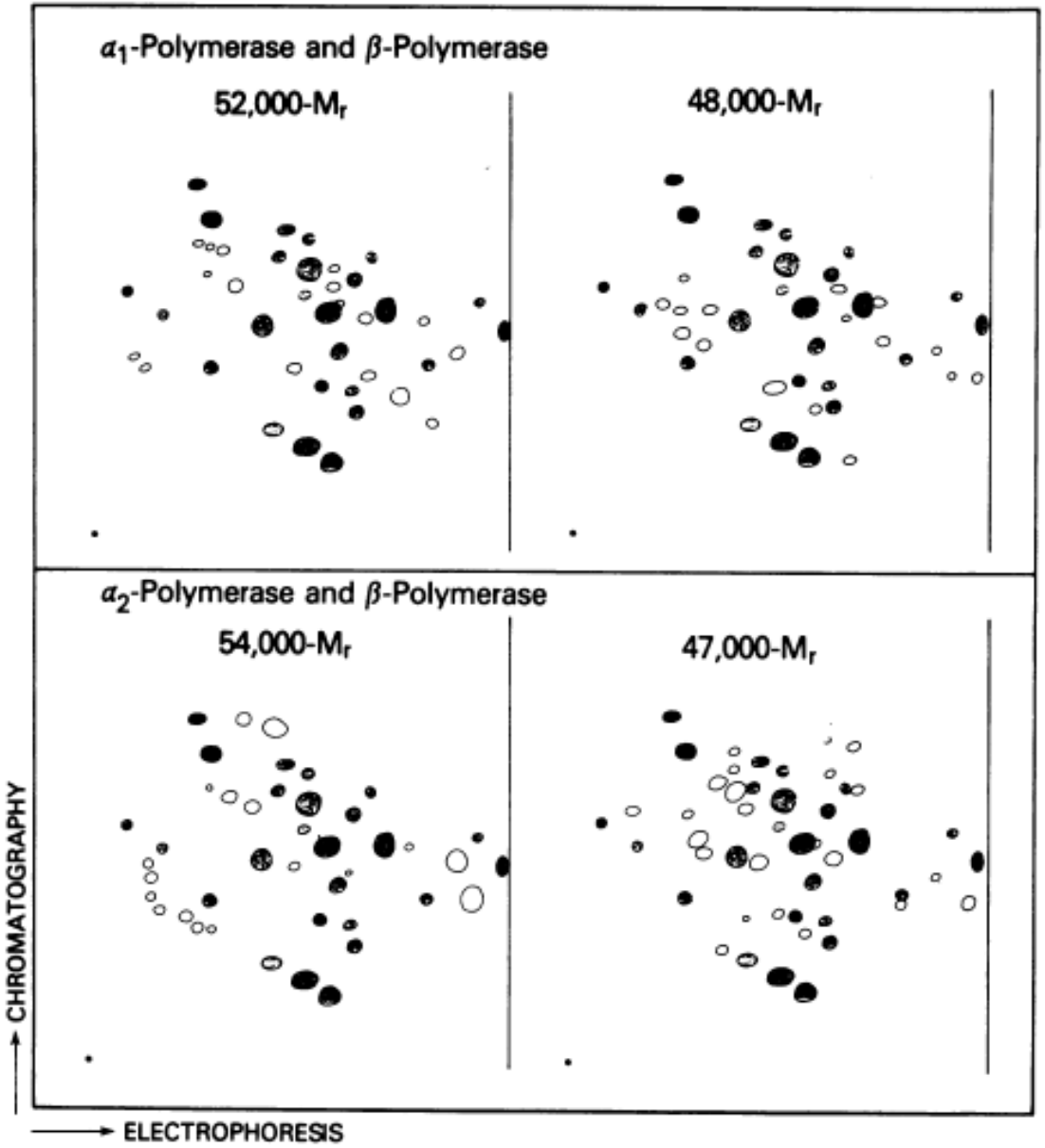
Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205, USA

Received 18 March 1980

ABSTRACT

Results presented here and in a previous paper (Tanabe et al. (1979) Biochemistry 18, 3401-3406) indicate that mouse β-polymerase is a single polypeptide with an apparent molecular weight of 40,000. This polypeptide has now been analyzed by tryptic peptide mapping. Comparison of the results with identical analysis of mouse α-polymerase reveals that the tryptic peptides derived from the two enzymes are different. These results indicate that β-polymerase is neither a subunit of α-polymerase nor a proteolytic degradation product of α-polymerase.

Peptide mapping from SDS-PAGE gel slices



39-kDa Pol ß tryptic peptides in black in each panel

Conclusion: Pol ß and Pol α are distinct at the primary structure level

Volume 10 Number 3 1982 **1982** Nucleic Acids Research

Structural homology among calf thymus α-polymerase polypeptides+

Calf thymus/ peptide mapping

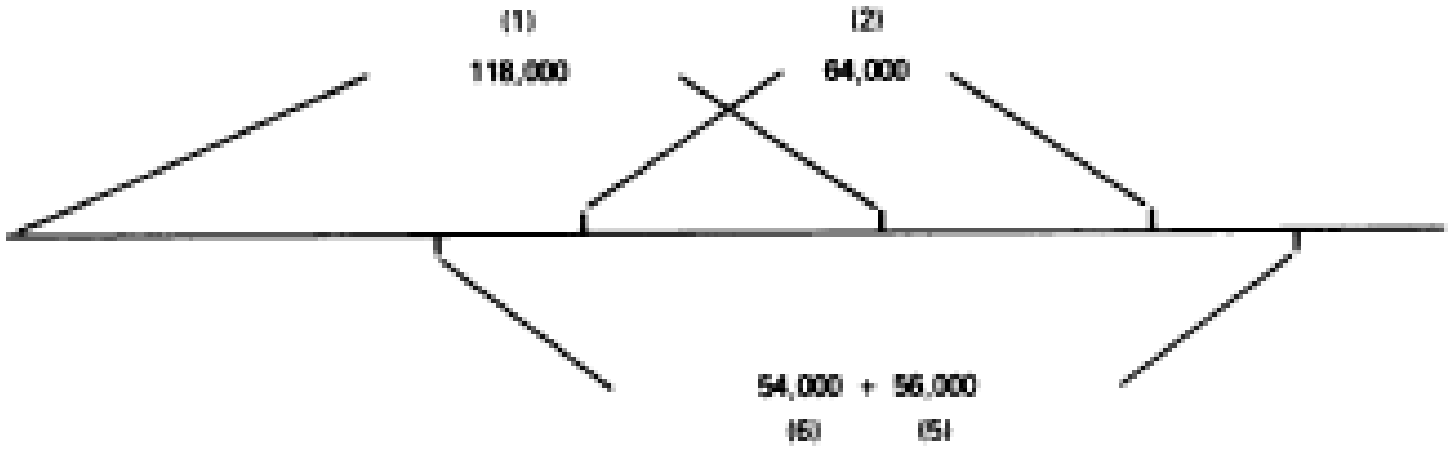
Waltraud Albert[1,2], Friedrich Grummt[2], Ulrich Hübscher[3] and Samuel H.Wilson[1]

[1]Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205, USA, [2]Institut für Biochemie, D-87 Wurzburg, FRG, and [3]Institut für Pharmakologie and Biochemie, Med.-Vet. Fakultät der Universität Zürich, Zürich, Switzerland

Received 22 October 1981; Revised and Accepted 17 December 1981

ABSTRACT

A sample of highly purified calf thymus α-polymerase contained an abundant 118,000-M_r polypeptide as well as five lower molecular weight polypeptides in the range of 54,000- to 64,000-M_r. This 118,000-M_r polypeptide was capable of DNA polymerase activity, as revealed by in situ assay after SDS-polyacrylamide gel electrophoresis. Tryptic peptide mapping indicated that the 118,000-M_r polypeptide shared extensive primary structure homology with 57,000-, 58,000- and 64,000-M_r polypeptides and some limited homology with 54,000- and 56,000-M_r polypeptides. This is the first evidence that lower and higher M_r polypeptides of purified calf thymus α-polymerase share sequence homology; these results are interpreted in the context of a model that predicts the existence of a common precursor with molecular weight >140,000.



Conclusion: Predicted M_r of catalytic subunit = ~180,000

ANALYTICAL BIOCHEMISTRY 135, 318–325 (1983) **1983**

Improved Conditions for Activity Gel Analysis of DNA Polymerase Catalytic Polypeptides

ESSAM KARAWYA, JUDITH A. SWACK, AND SAMUEL H. WILSON

Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

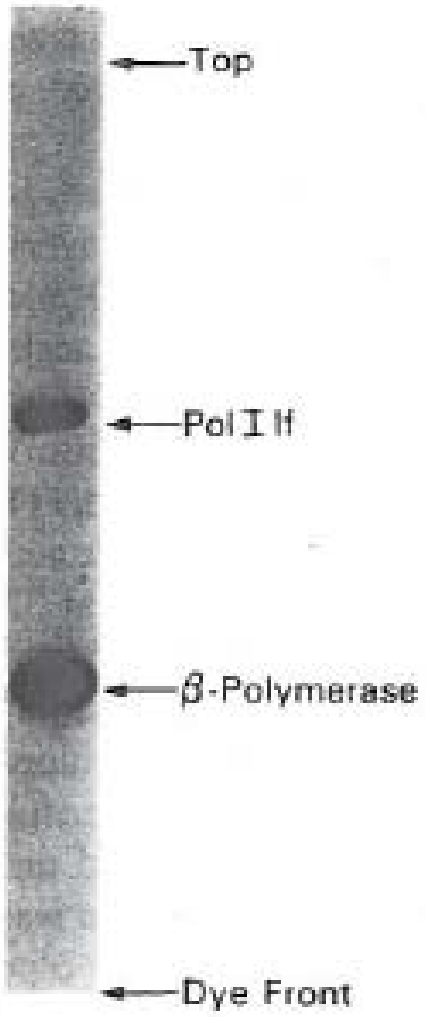
Received April 25, 1983

In a study of mouse DNA polymerase catalytic polypeptides using activity gel analysis, it was found that the sensitivity of detection of purified enzymes is markedly increased by addition of a heterogeneous mixture of proteins to the enzyme sample prior to electrophoresis (Karawya E., and Wilson, S. H. (1982) *J. Biol. Chem.* **257,** 13,129–13,134). This modification and the use of a micromolar level of [^{32}P]dNTP substrate are the basis of an improved activity gel assay for DNA polymerase catalytic polypeptides. This modified assay is several orders of magnitude more sensitive than the original procedure (Spanos, A., Sedgwick, S. G., Yarranton, G. T., Hubscher, U., and Banks, G. R. (1981) *Nucl. Acids Res.* **9,** 1825–1839), and it enables measurement of two reference enzymes, calf β-polymerase and *Escherichia coli* DNA polymerase I large fragment, in the picogram range. Further, it was found that it is essential to survey different lots of sodium dodecyl sulfate to identify those which enable high enzyme activity signals after renaturation.

KEY WORDS: *in situ* DNA polymerase assay; SDS–polyacrylamide gel electrophoresis.

SDS-PAGE, as usual, but with DNA in the gel; then, renature in a DNA polymerase reaction mixture containing ^{32}P-labeled dNTPs. Wash and conduct autoradiography.

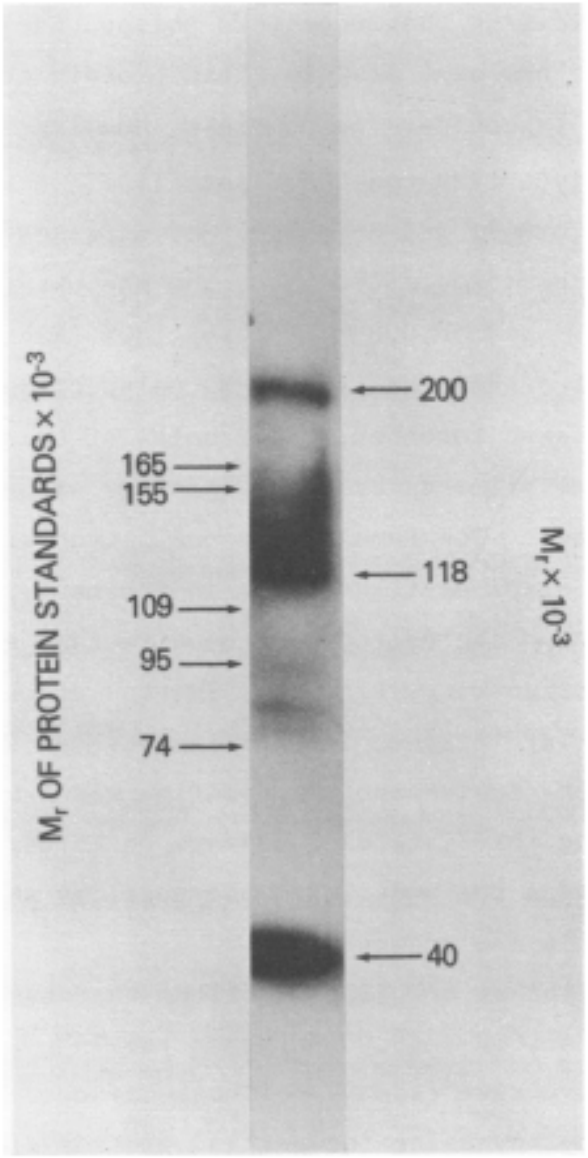
Activity gel (SDS-PAGE) analysis with purified enzymes (positive controls)



Klenow

Pol ß

Activity gel analysis of calf thymus crude extract



Pol α (?)

?

Pol ß

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7777–7781, December 1984
Biochemistry

1984

Identification of a higher molecular weight DNA polymerase α catalytic polypeptide in monkey cells by monoclonal antibody

(mammalian DNA polymerase α/renaturation of enzyme)

ESSAM KARAWYA*, JUDITH SWACK*, WALTRAUD ALBERT*†, JOSEPH FEDORKO‡, JOHN D. MINNA‡, AND SAMUEL H. WILSON*

*Laboratory of Biochemistry and ‡National Cancer Institute-Navy Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205; and †Institut für Biochemie, Universitat Wurzburg, Wurzburg, Federal Republic of Germany

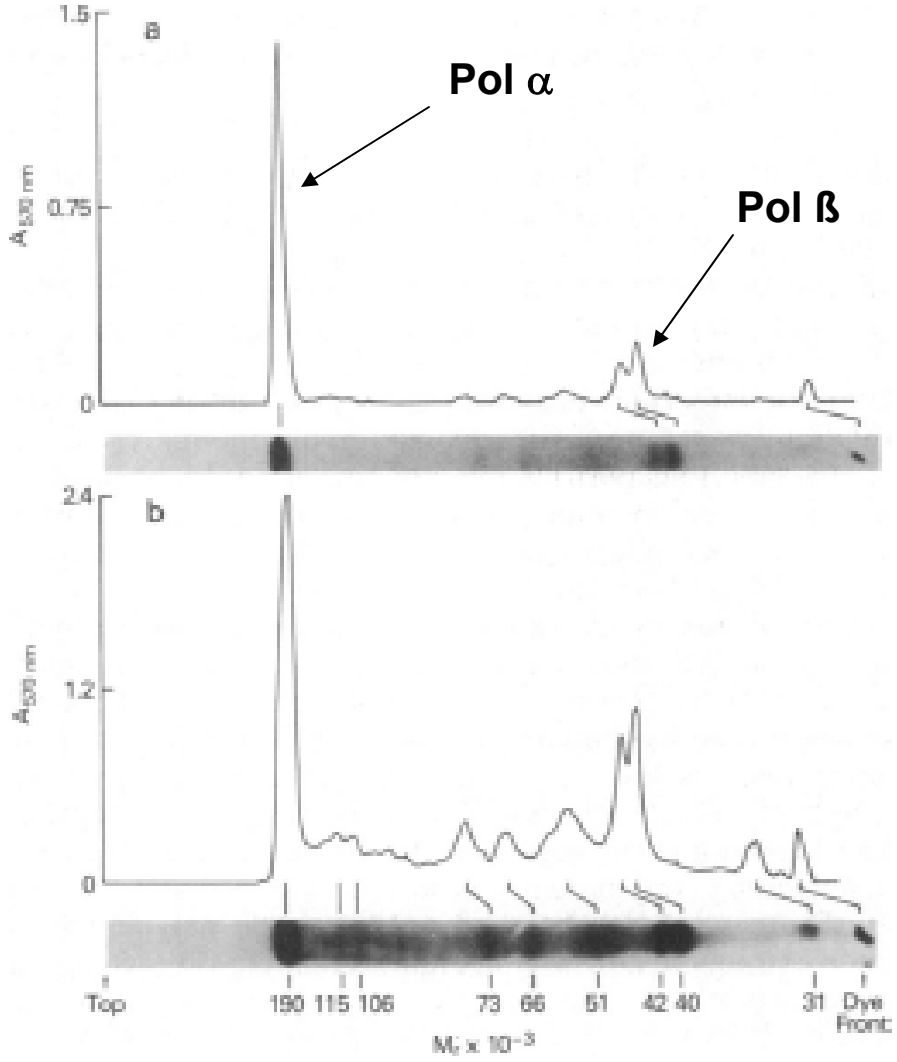
Communicated by Maxine Singer, August 28, 1984

ABSTRACT A monoclonal antibody against purified calf DNA polymerase α (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) was used to immunoprecipitate proteins from a crude soluble extract of growing monkey BSC-1 cells. Immunoprecipitates contained familiar DNA polymerase α catalytic polypeptides of M_rs ≈ 115,000 and 70,000 and also a M_r 40,000 catalytic polypeptide; the major component in the immunoprecipitates, however, was a polypeptide of M_r ≈ 190,000 not previously identified as a DNA polymerase. This protein was capable of DNA polymerase activity after electroelution from NaDodSO$_4$/polyacrylamide gels and renaturation. The highly purified enzyme so obtained was active with poly(dT)·oligo(rA) as template·primer, resistant to dideoxy TTP (ddTTP), and inhibited by aphidicolin and butylphenyldeoxyguanosine 5′-triphosphate, thus identifying it as a DNA polymerase α. The results indicate that a polypeptide of M_r ≈ 190,000 is an abundant component among DNA polymerase α catalytic polypeptides in growing monkey cells.

IP with monoclonal ab. using extract from ^{35}S-labeled cells.

Renature ~190,000 peptide from SDS-PAGE gel slice.

Monoclonal antibody IP of extract from ^{35}S-labeled cells



Brief exposure

Longer exposure

?

Pol alpha activity found in the ~190,000 peptide

Table 1. DNA polymerase activity of renatured proteins after electroelution from $NaDodSO_4$/polyacrylamide gels

Protein electrophoresed	Gel region electroeluted, $M_r \times 10^{-3}$	DNA polymerase activity recovered, milliunits* Exp. 1	Exp. 2
Immunoprecipitates of BSC-1 cell extract†	>190	0.07	—
	190 ± 9	0.30	0.37
	180–120	0.02	—
	115 ± 4	0.02	0.07
	110–75	0.04	—
	70 ± 4	0.03	0.02
	65–43	0.02	—
	40 ± 2	0.60	0.15
		Total 1.10	0.61
Nonimmune IgM	190 ± 9	0	
	115 ± 4	0	
	70 ± 4	0	
	40 ± 2	0	
Reference DNA polymerases			
Partially purified calf thymus α-polymerase (20 milliunits)‡	190 ± 9	0.38	0.6
	180–120	0.32	—
	115 ± 4	0.19	1.02
	110–75	0.30	0.21
	70 ± 4	0.23	1.42
	65–43	0.26	—
	40 ± 2	0.22	0.1
		Total 1.90	3.35

Activity

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i) Identification by interaction with BER intermediate and MS
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Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 5106–5110, July 1986
Biochemistry

1986

Structure of rat DNA polymerase β revealed by partial amino acid sequencing and cDNA cloning*

(gt11/tryptic peptide sequencing/mRNA·cDNA hybridization/protein secondary structure predictions)

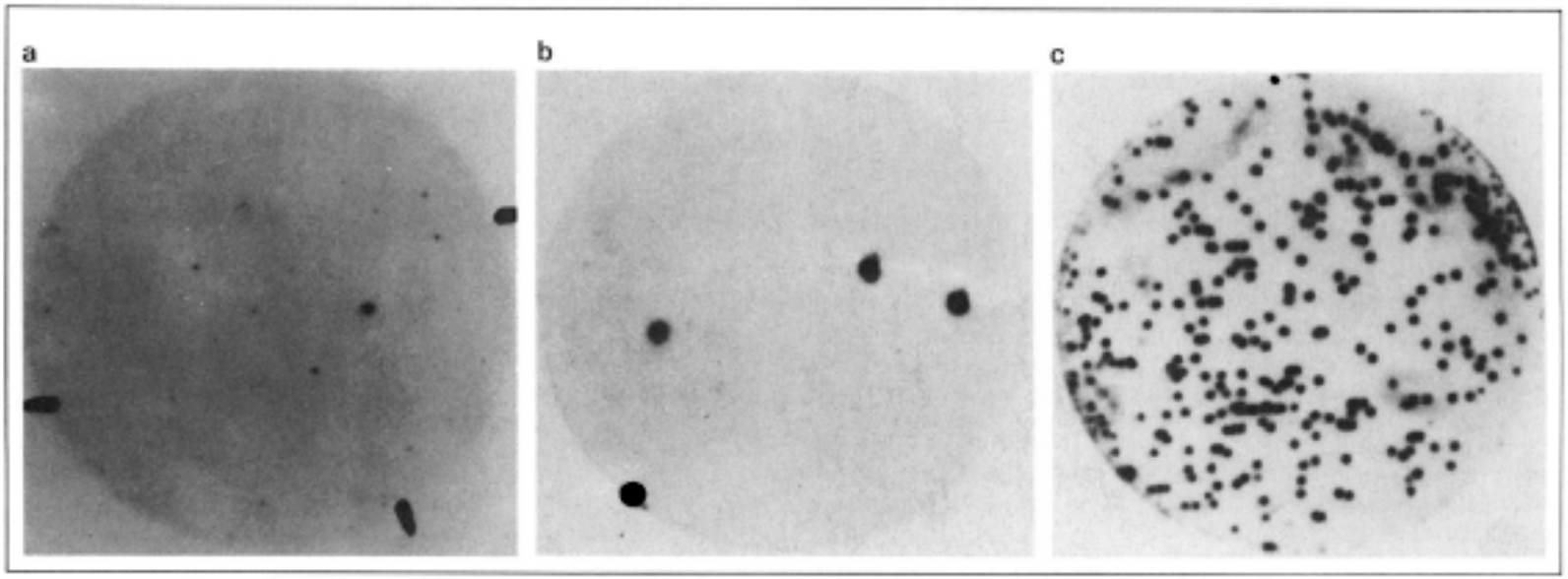
B. Z. ZMUDZKA†, D. SENGUPTA†, A. MATSUKAGE‡, F. COBIANCHI†, P. KUMAR†, AND S. H. WILSON†‡

†Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and ‡Aichi Cancer Center Research Institute, Nagoya 464, Japan

Communicated by Mahlon Hoagland, April 4, 1986

ABSTRACT **A cDNA library of newborn rat brain poly(A)$^+$ RNA in phage λgt11 was screened with a polyclonal antibody against chicken DNA polymerase β. One positive phage was isolated and purified after testing 2×10^7 recombinants. This phage, designated λpolβ-10, contained an 1197-base-pair cDNA insert that corresponded to a mRNA with a poly(A) sequence at the 3′ terminus and a single, long open-reading frame of 957 bases. The open-reading frame, starting 44 residues from the 5′ end of the cDNA, predicted a 36,375-Da protein of 318 amino acids. Comparison of this deduced amino acid sequence with the partial sequence obtained with purified polymerase β revealed a match of six tryptic peptides, involving a total of 47 amino acid residues. This confirmed the identity of the cDNA. Blot-hybridization analysis of newborn rat brain poly(A)$^+$ RNA revealed a mRNA species of approximately the same size as the cDNA insert; in addition, a second mRNA species ≈4000 bases long was detected. Computer-derived secondary structure analysis of the enzyme predicted seven regions of α-helix distributed throughout and three regions of β-sheet.**

Example of phage λ plaque purification



Vol. 136, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1986 Pages 341-347

SEQUENCE OF HUMAN DNA POLYMERASE β mRNA OBTAINED THROUGH cDNA CLONING

D.N. SenGupta, B.Z. Zmudzka, P. Kumar, F. Cobianchi, J. Skowronski and S.H. Wilson

Laboratory of Biochemistry, National Cancer Institute
National Institutes of Health, Bethesda, MD 20892

Received March 6, 1986

Summary: A cDNA library from polyA$^+$ RNA of a human teratocarcinoma cell line in phage λgt11 was screened with a fragment of the rat β-polymerase cDNA, λpolβ-10, as probe. Five positive phage were identified and plaque purified. The cDNA of one positive clone selected for detailed study was 1257 bp. This insert was sequenced and found to contain the coding region for β-polymerase, as well as 163 bp and 137 bp from the 5' and 3' untranslated regions, respectively. The primary structure of human β-polymerase (318 amino acids, M_r=36,133) deduced from the cDNA was similar to rat β-polymerase (95% matched residues). The greatest difference between the sequences of the human and rat cDNAs was in the 3' untranslated regions (64% matched base residues). These results provide necessary sequence information for study of the human β-polymerase gene.

DNA Replication and Mutagenesis
Edited by R. E. Moses and W. C. Summers

1988

Chapter 6

Human DNA Polymerase β

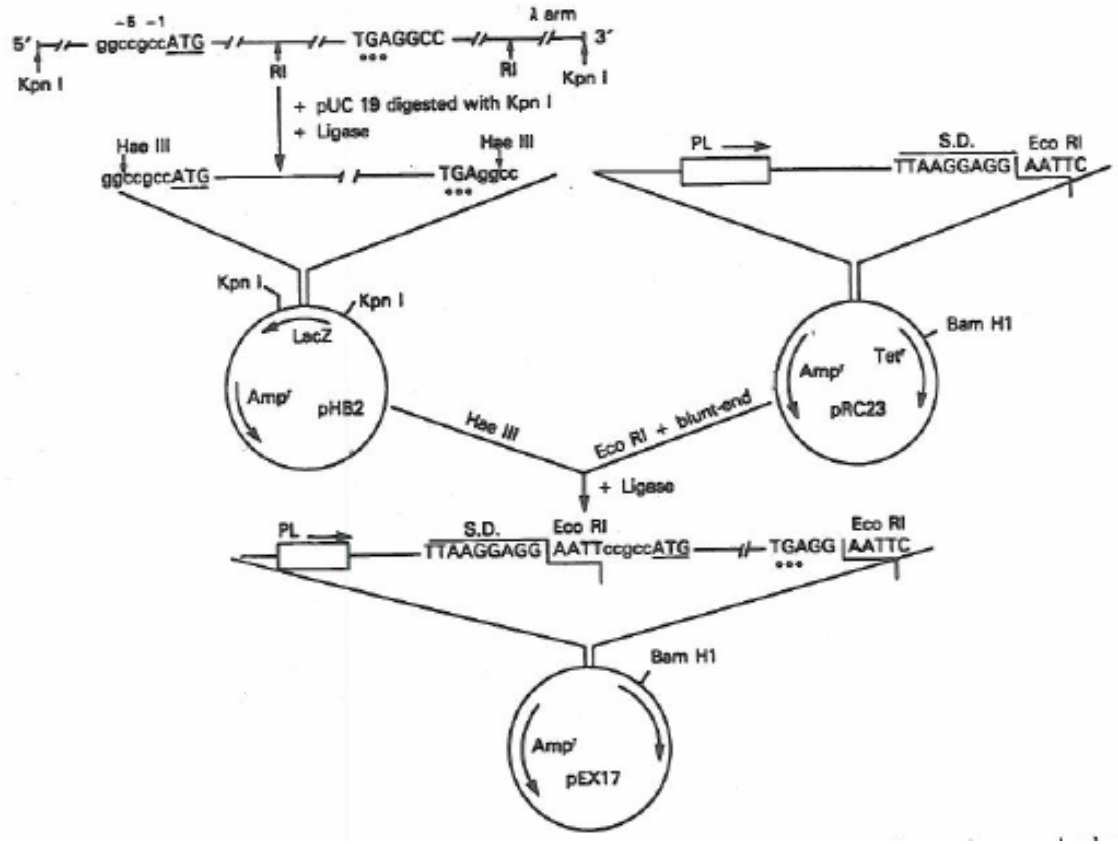
Expression in *Escherichia coli* and Characterization of the Recombinant Enzyme

John Abbotts, Dibyendu N. SenGupta, Barbara Z. Zmudzka, Steven G. Widen, and Samuel H. Wilson

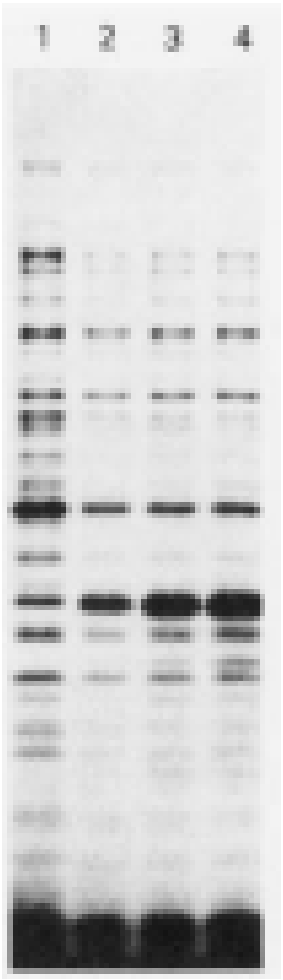
DNA polymerase β, a DNA repair polymerase of eucaryotic cells (for a review, see reference 6), is seen as a model enzyme for structure-function analysis of the nucleotidyltransferase reaction by DNA polymerases (21). This enzyme is the simplest DNA polymerase known in both size and catalytic repertoire. It is also the least accurate of eucaryotic polymerases, showing misinsertion error rates of 1/1,300 to 1/6,600 on natural DNA templates (9, 12). This suggests that DNA repair is a mutagenic process or that auxiliary cellular factors must enhance the fidelity of DNA repair synthesis. The human and rat β-polymerases are polypeptides of 335 amino acids, and secondary structure predictions suggest an ordinary globular structure with a high α-helix content (19, 27). The purified enzyme lacks exonuclease activities and detectable reverse reactions (5, 21), and the polymerase activity is fully distributive under most reaction conditions (2). Thus, the β-polymerase mechanism is a two-substrate, two-product reaction and follows ordered BiBi kinetics (21).

To examine physical biochemical properties and structure-function relationships of mammalian β-polymerase, we overexpressed the coding region of a human β-polymerase cDNA (19) in the λ p_L promoter-based bacterial expression system pRC23 (1) and purified the recombinant enzyme in milligram quantities. Studies revealed that the enzyme is a characteristic β-polymerase and is appropriate for structure-function studies of this enzyme. Here we describe the expression and characterization of the recombinant polymerase and discuss opportunities for further investigation of the enzyme and its role in DNA repair.

John Abbotts, Dibyendu N. SenGupta, Barbara Z. Zmudzka, Steven G. Widen, and Samuel H. Wilson • Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20892.



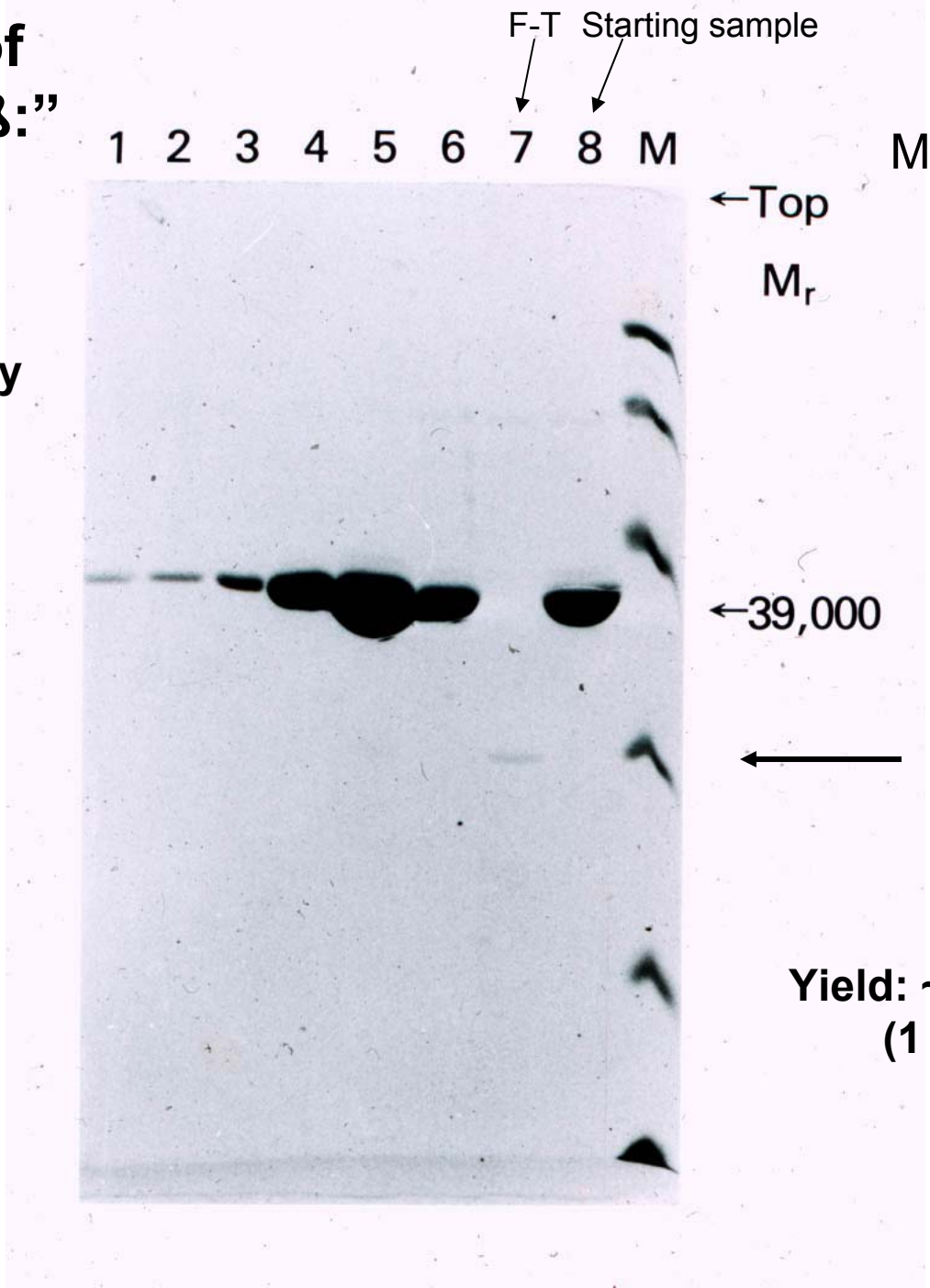
Induction@ 42°C
1h 2h 3h



Example with rat enzyme

“Polishing of purified Pol ß:”

Routine FPLC chromatography (1987)

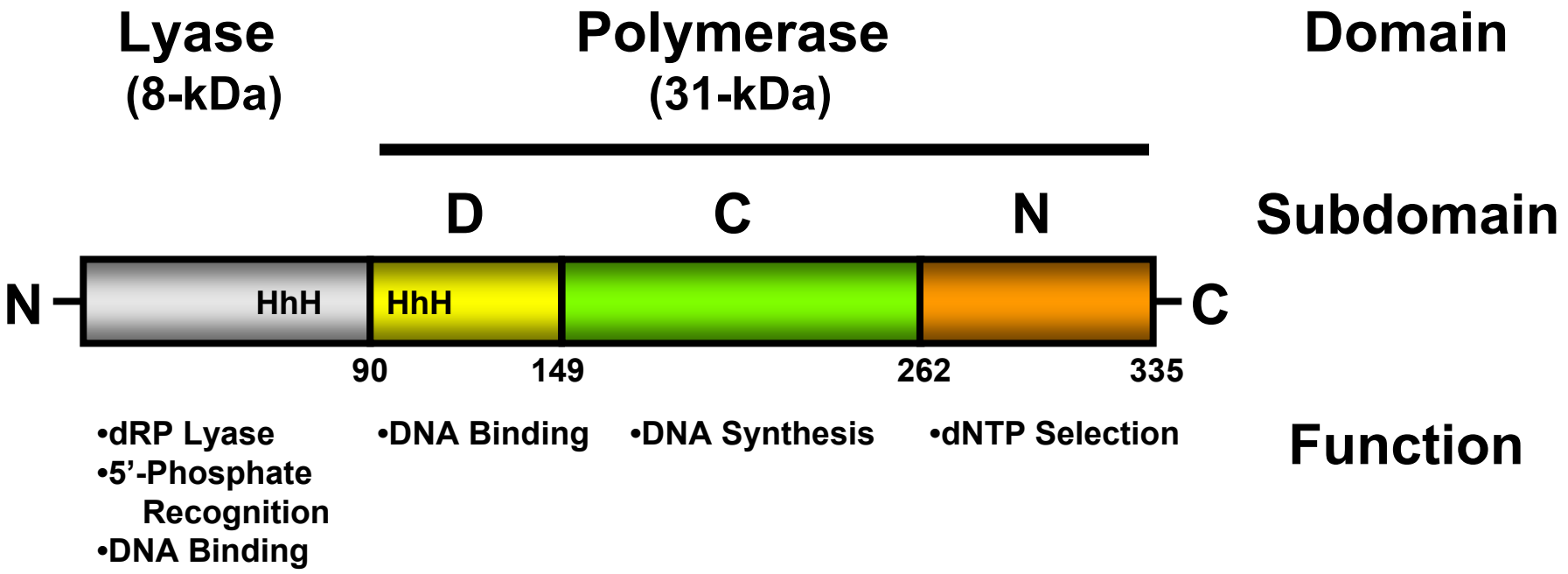


M= 10 µg each

Yield: ~100 mg per batch (1 liter culture) !

DNA Polymerase ß

(335 residues; 39 kDa)



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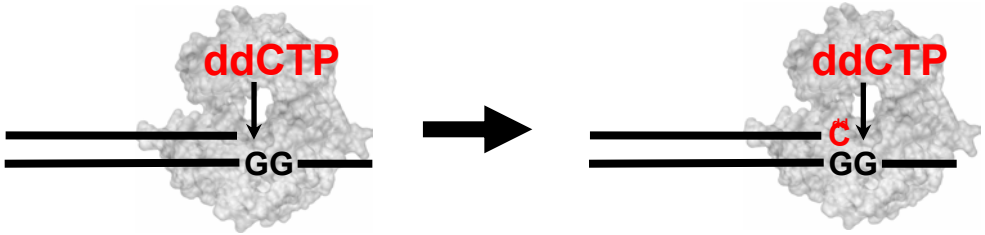
Ternary Complex Structure

(collaboration with Joe Kraut, Huguette Pelletier & Mike Sawaya)

Structure of substrate complex: Enzyme; oligonucleotide DNA; and correct incoming dNTP

Polymerase + DNA + ddCTP/Mg^{2+}



Trapped intermediate with ddCMP at the primer terminus.

(Project started in 1987 with phone call from Joe Kraut)

RESEARCH ARTICLE

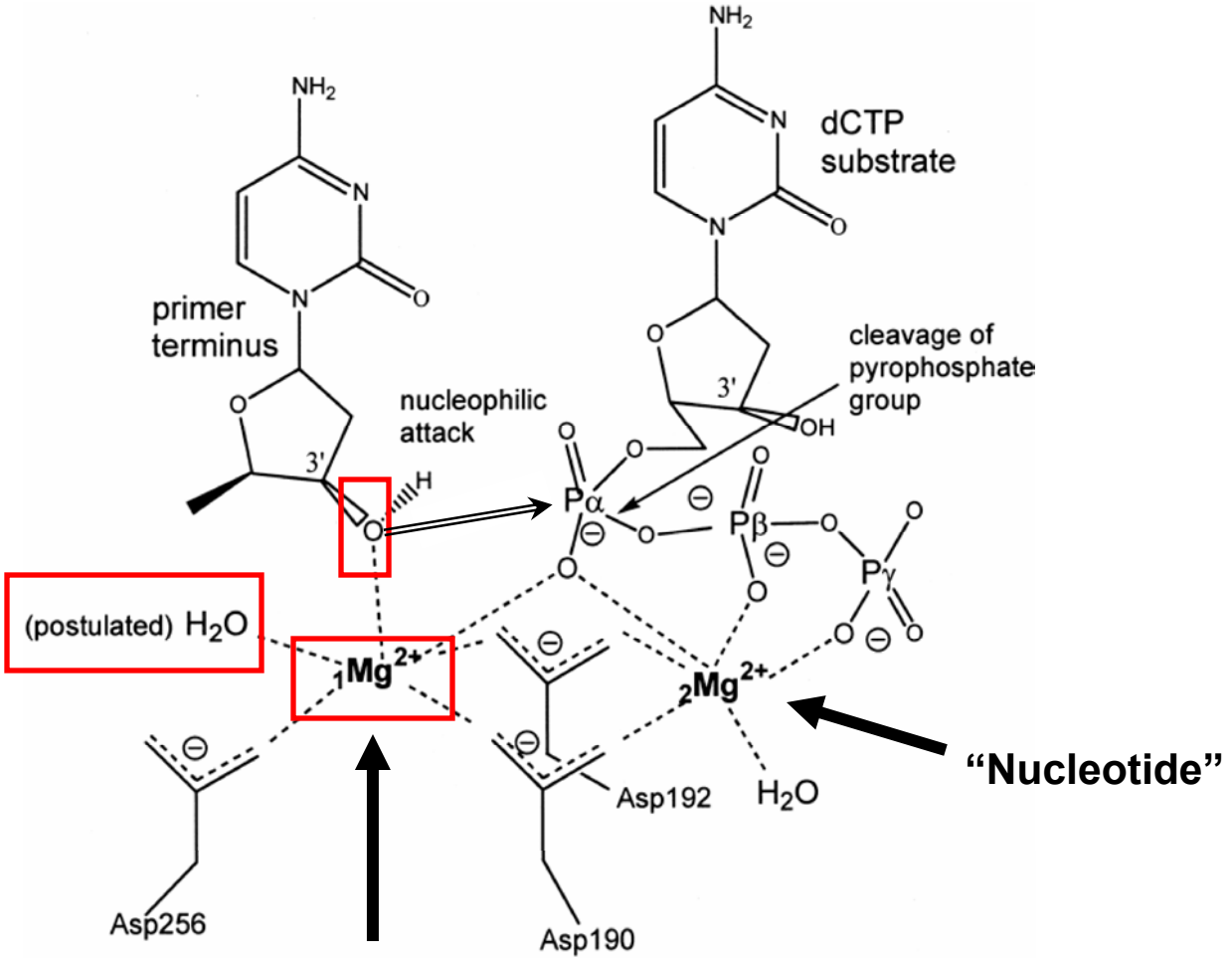
Science, 264:1891-1903, 1994

Structures of Ternary Complexes of Rat DNA Polymerase β, a DNA Template-Primer, and ddCTP

Huguette Pelletier, Michael R. Sawaya, Amalendra Kumar, Samuel H. Wilson, Joseph Kraut

Two ternary complexes of rat DNA polymerase β (pol β), a DNA template-primer, and dideoxycytidine triphosphate (ddCTP) have been determined at 2.9 Å and 3.6 Å resolution, respectively. ddCTP is the triphosphate of dideoxycytidine (ddC), a nucleoside analog that targets the reverse transcriptase of human immunodeficiency virus (HIV) and is at present used to treat AIDS. Although crystals of the two complexes belong to different space groups, the structures are similar, suggesting that the polymerase-DNA-ddCTP interactions are not affected by crystal packing forces. In the pol β active site, the attacking 3′-OH of the elongating primer, the ddCTP phosphates, and two Mg^{2+} ions are all clustered around Asp^{190}, Asp^{192}, and Asp^{256}. Two of these residues, Asp^{190} and Asp^{256}, are present in the amino acid sequences of all polymerases so far studied and are also spatially similar in the four polymerases—the Klenow fragment of *Escherichia coli* DNA polymerase I, HIV-1 reverse transcriptase, T7 RNA polymerase, and rat DNA pol β—whose crystal structures are now known. A two-metal ion mechanism is described for the nucleotidyl transfer reaction and may apply to all polymerases. In the ternary complex structures analyzed, pol β binds to the DNA template-primer in a different manner from that recently proposed for other polymerase-DNA models.

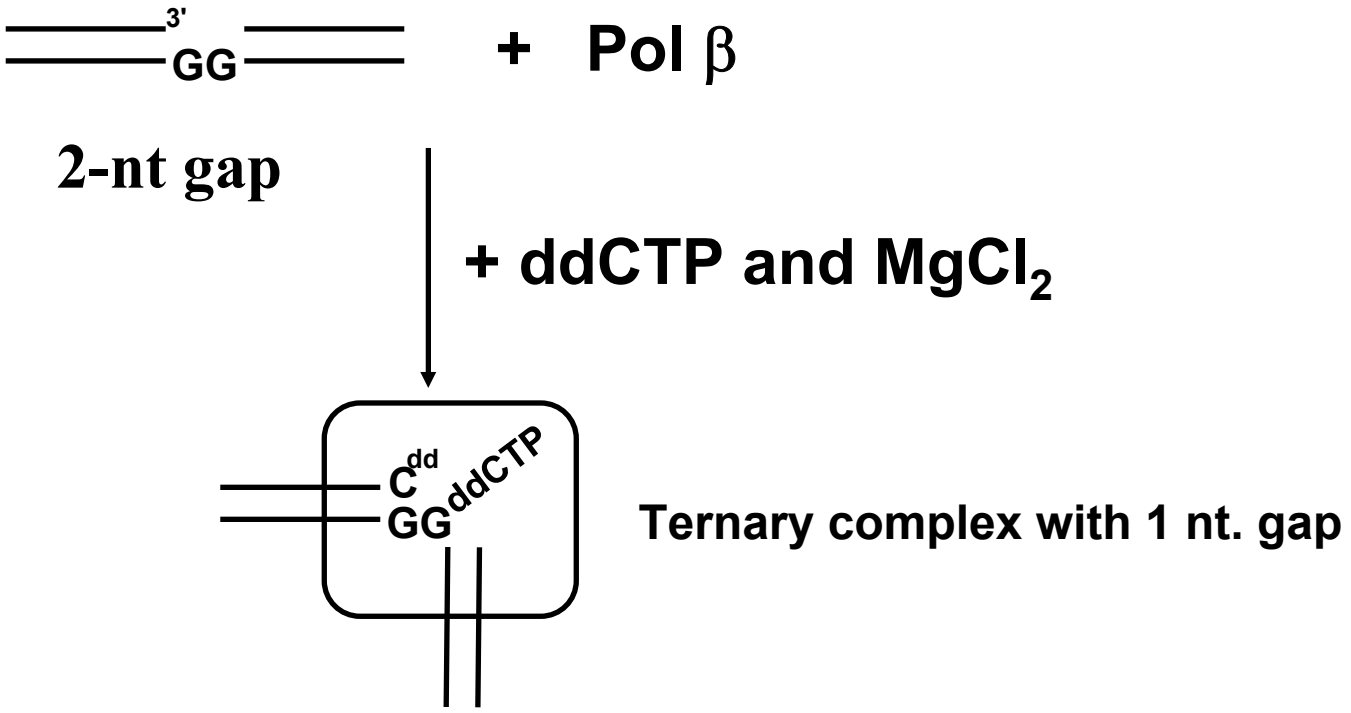
Model for pre-catalytic complex (atoms in red postulated)



Characterizing BER DNA synthesis using crystallography

(Sawaya et al., Biochemistry, 36:11205-11215, 1997)

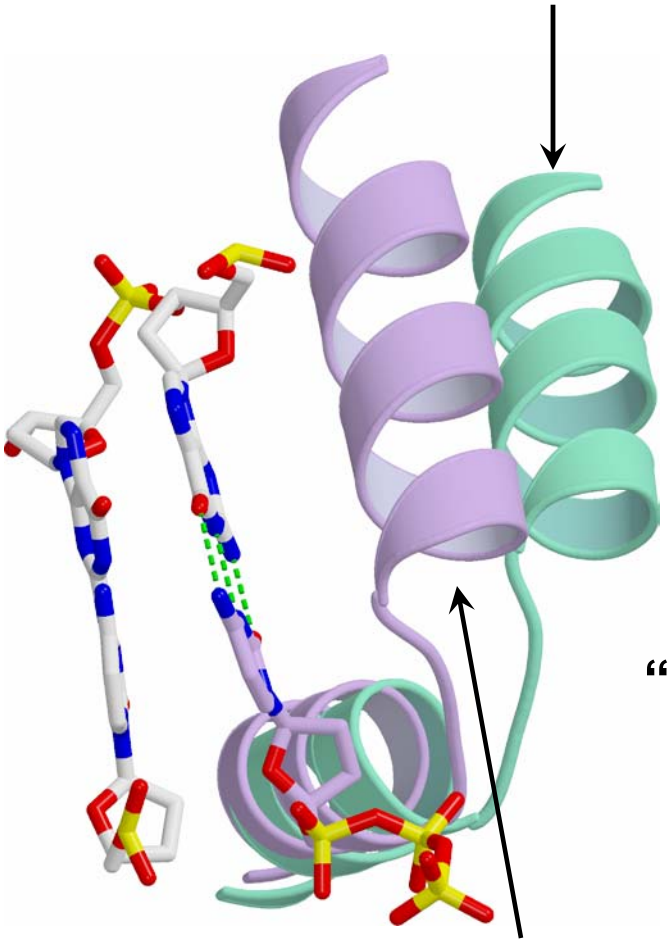
Gapped and nicked BER intermediates used as DNA substrate



(Also obtained binary complexes with nicked DNA)

Local Conformational Change Upon Formation of a Ternary Complex with a Watson-Crick Base Pair

Binary DNA Complex: OPEN



α-helix N

"Induced fit" hypothesis for DNA synthesis

Ternary Complex: CLOSED

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THE JOURNAL OF BIOLOGICAL CHEMISTRY

1995

Vol. 270, No. 2, Issue of January 13, pp. 949–957, 1995
Printed in U.S.A.

DNA Polymerase β Conducts the Gap-filling Step in Uracil-initiated Base Excision Repair in a Bovine Testis Nuclear Extract*

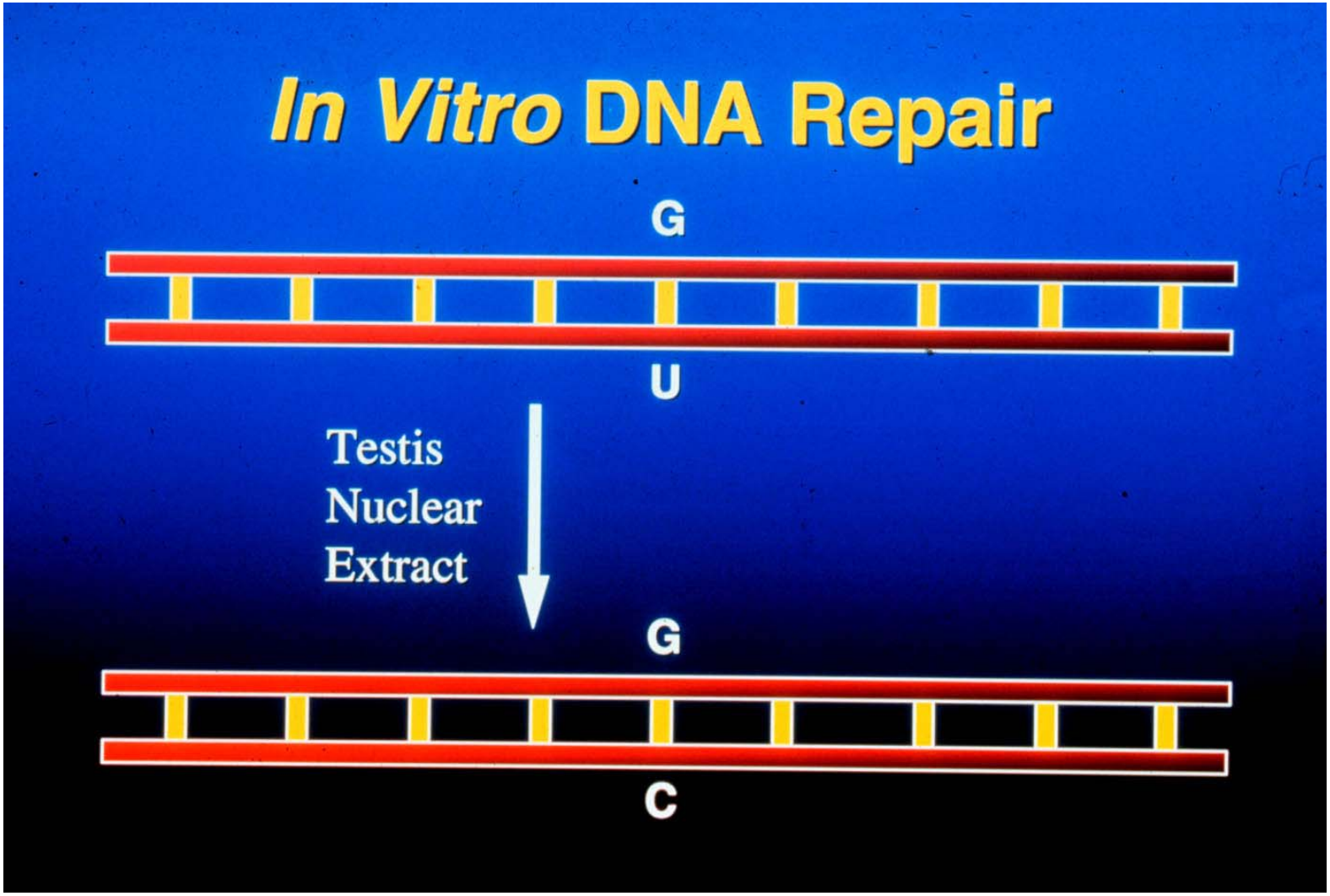
(Received for publication, August 5, 1994, and in revised form, October 26, 1994)

Rakesh K. Singhal, Rajendra Prasad, and Samuel H. Wilson‡

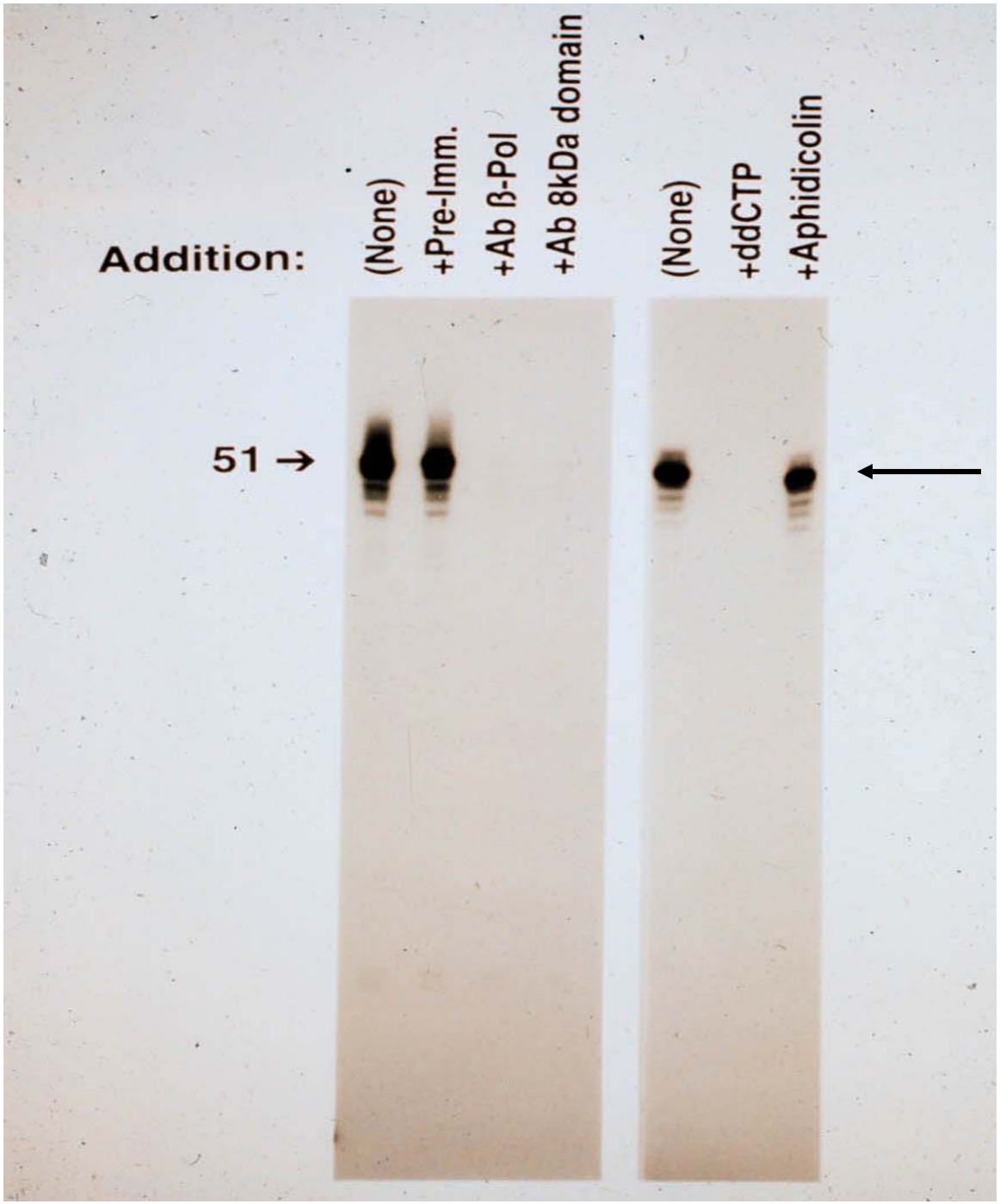
From the Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1068

The G:U mismatch in genomic DNA mainly arises from deamination of cytosine residues and is repaired by the base excision repair pathway. We found that a bovine testis crude nuclear extract conducts uracil-initiated base excision repair *in vitro*. A 51-base pair synthetic DNA substrate containing a single G:U mismatch was used, and incorporation of dCMP during repair was exclusively to replace uracil. A neutralizing polyclonal antibody against DNA polymerase β (β-pol) inhibited the repair reaction. ddCTP also inhibited the repair reaction, whereas aphidicolin had no significant effect, suggesting that activity of β-pol was required. Next, the base excision repair system was reconstituted using partially purified components. Several of the enzymatic activities required were resolved, such that DNA ligase and the uracil-DNA glycosylase/apurinic/apyrimidinic endonuclease activities were separated from the DNA polymerase requirement. We found that purified β-pol could restore full DNA repair activity to the DNA polymerase-depleted fraction, whereas purified DNA polymerases α, δ, and ε could not. These results with purified proteins corroborated results obtained with the crude extract and indicate that β-pol is responsible for the single-nucleotide gap filling reaction involved in this *in vitro* base excision repair system.

Extract mediated Uracil-DNA BER and use of a neutralizing polyclonal antibodies to Pol ß



^{32}P-labeled dCMP incorporation into a 51-bp oligonucleotide product molecule


Addition:
(None)
+Pre-Imm.
+Ab β-Pol
+Ab 8kDa domain
(None)
+ddCTP
+Aphidicolin
51 →
Repair Product

Requirement of mammalian DNA polymerase-β in base-excision repair

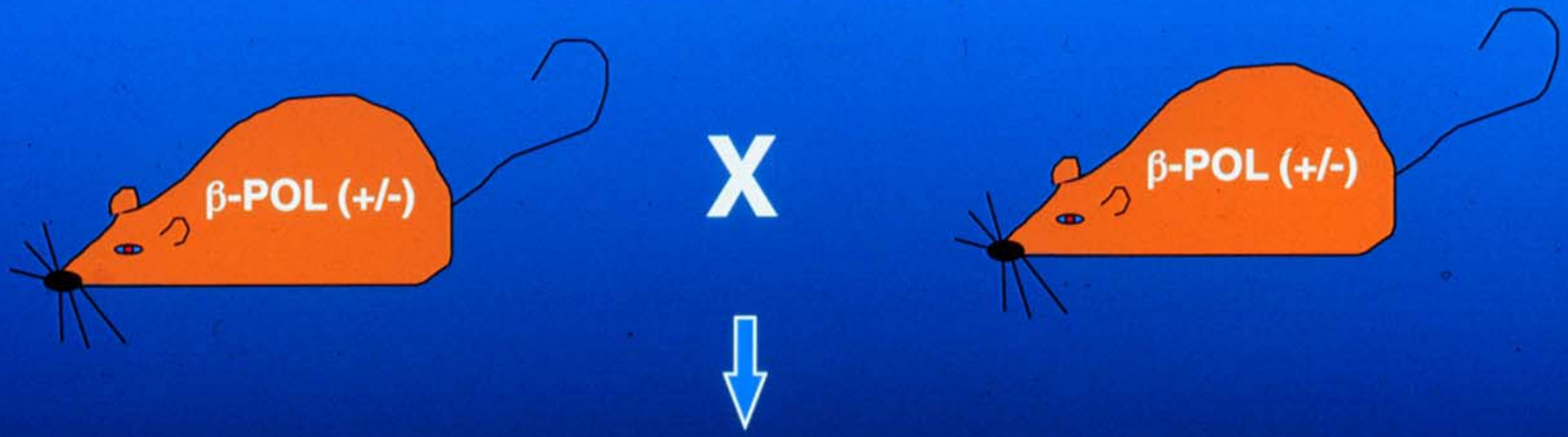
Robert W. Sobol*, Julie K. Horton†, Ralf Kühn‡, Hua Gu‡§, Rakesh K. Singhal*, Rajendra Prasad*, Klaus Rajewsky‡ & Samuel H. Wilson*||

* Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1068, USA
† Laboratory of Cell Biology, Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1068, USA
‡ Institute of Genetics, University of Cologne, Weyertal 121, D-50931 Cologne, Germany

SYNTHESIS of DNA by DNA polymerase-β is distributive on single-stranded DNA templates, but short DNA gaps with a 5′ PO_4 in the gap are filled processively to completion[1,2]. *In vitro* studies have suggested a role of β-polymerase in different types of DNA repair[3–9]. However, the significance of these studies to the *in vivo* role of β-polymerase has remained unclear. Because genetic studies are essential for determining the physiological role of a gene, we established embryonic fibroblast cell lines homozygous for a deletion mutation in the gene encoding DNA polymerase-β. Extracts from these cell lines were found to be defective in uracil-initiated base-excision repair. The β-polymerase-deleted cells are normal in viability and growth characteristics, although they exhibit increased sensitivity to monofunctional DNA-alkylating agents, but not to other DNA-damaging agents. Both the deficiency in base-excision repair and hypersensitivity to DNA-alkylating agents are rescued following stable transfection with a wild-type β-polymerase minitransgene. These studies demonstrate that β-polymerase functions specifically in base-excision repair *in vivo*.

Nature, 379:183-186, 1996

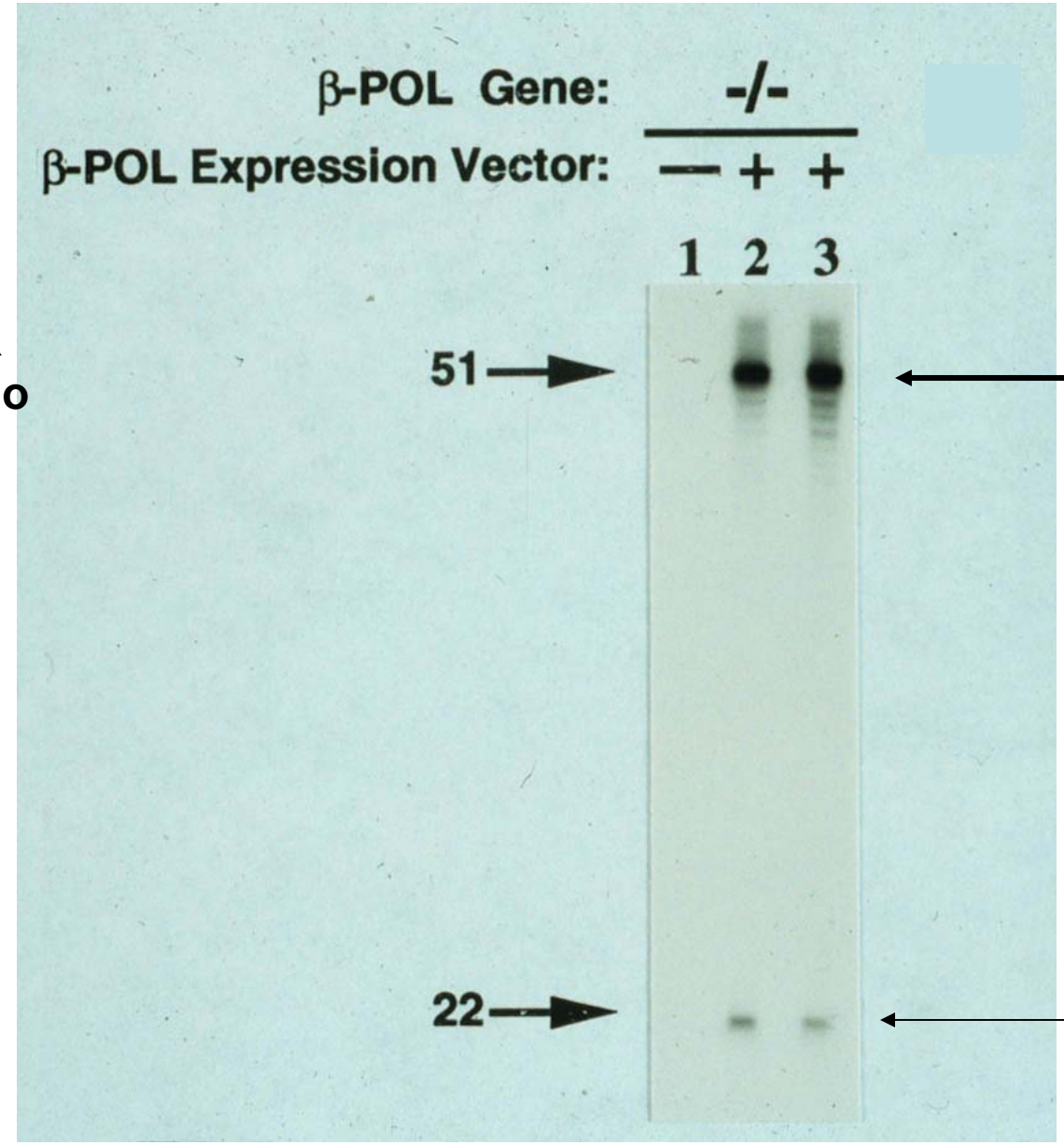
ISOLATION OF EMBRYONIC FIBROBLAST CELLS EXHIBITING A β-POL KNOCKOUT



Live Born	Embryos (10-12 days)
β-pol (+/+)	β-pol (+/+)
β-pol (+/-)	β-pol (+/-)
	β-pol (-/-)

Complementation in Pol ß -/- MEF line with cDNA expression vector

Uracil-DNA BER using 51-bp oligo as substrate: U at position 22.



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Enzyme-DNA Interactions Required for Efficient Nucleotide Incorporation and Discrimination in Human DNA Polymerase β*

(Received for publication, March 8, 1996)

William A. Beard‡, Wendy P. Osheroff§, Rajendra Prasad‡, Michael R. Sawaya¶, Madhuri Jaju‡, Thomas G. Wood‡, Joseph Kraut¶, Thomas A. Kunkel§, and Samuel H. Wilson‡‖

From the ‡Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1068, the §Laboratory of Molecular Genetics, NIEHS, Research Triangle Park, North Carolina 27709, and the ¶Department of Chemistry, University of California, San Diego, California 92093-0317

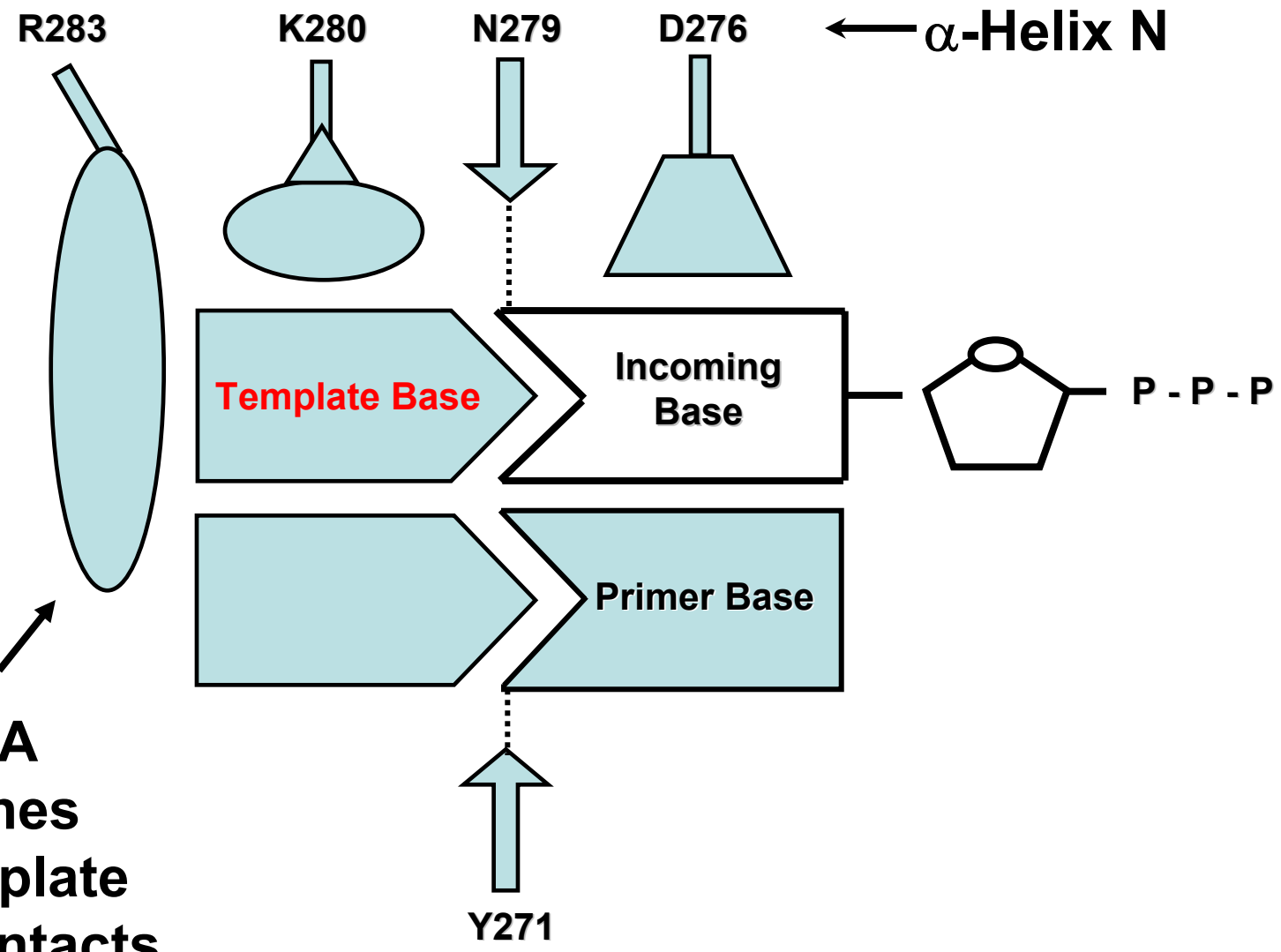
In the crystal structure of a substrate complex, the side chains of residues Asn^{279}, Tyr^{271}, and Arg^{283} of DNA polymerase β are within hydrogen bonding distance to the bases of the incoming deoxynucleoside 5′-triphosphate (dNTP), the terminal primer nucleotide, and the templating nucleotide, respectively (Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., and Kraut, J. (1994) *Science* 264, 1891–1903). We have altered these side chains through individual site-directed mutagenesis. Each mutant protein was expressed in *Escherichia coli* and was soluble. The mutant enzymes were purified and characterized to probe their role in nucleotide discrimination and catalysis. A reversion assay was developed on a short (5 nucleotide) gapped DNA substrate containing an opal codon to assess the effect of the amino acid substitutions on fidelity. Substitution of the tyrosine at position 271 with phenylalanine or histidine did not influence catalytic efficiency (k_{cat}/K_m) or fidelity. The hydrogen bonding potential between the side chain of Asn^{279} and the incoming nucleotide was removed by replacing this residue with alanine or leucine. Although catalytic efficiency was reduced as much as 17-fold for these mutants, fidelity was not. In contrast, both catalytic efficiency and fidelity decreased dramatically for all mutants of Arg^{283} (Ala > Leu > Lys). The fidelity and catalytic efficiency of the alanine mutant of Arg^{283} decreased 160- and 5000-fold, respectively, relative to wild-type enzyme. Sequence analyses of the mutant DNA resulting from short gap-filling synthesis indicated that the types of base substitution errors produced by the wild-type and R283A mutant were similar and indicated misincorporations resulting in frequent T·dGTP and A·dGTP mispairing. With R283A, a dGMP was incorporated opposite a template thymidine as often as the correct nucleotide. The x-ray crystallographic structure of the alanine mutant of Arg^{283} verified the loss of the mutated side chain. Our results indicate that specific interactions between DNA polymerase β and the template base, but *not* hydrogen bonding to the incoming dNTP or terminal primer nucleotide, are required for both high catalytic efficiency and nucleotide discrimination.

Role of Arg283 in catalysis and fidelity

R283A is deficient in catalytic efficiency and in fidelity. Explanation through crystal structure analysis.

JBC, 271:12213-12220, 1996

“ Tight fit for new base pair”



R283A abolishes key template strand contacts

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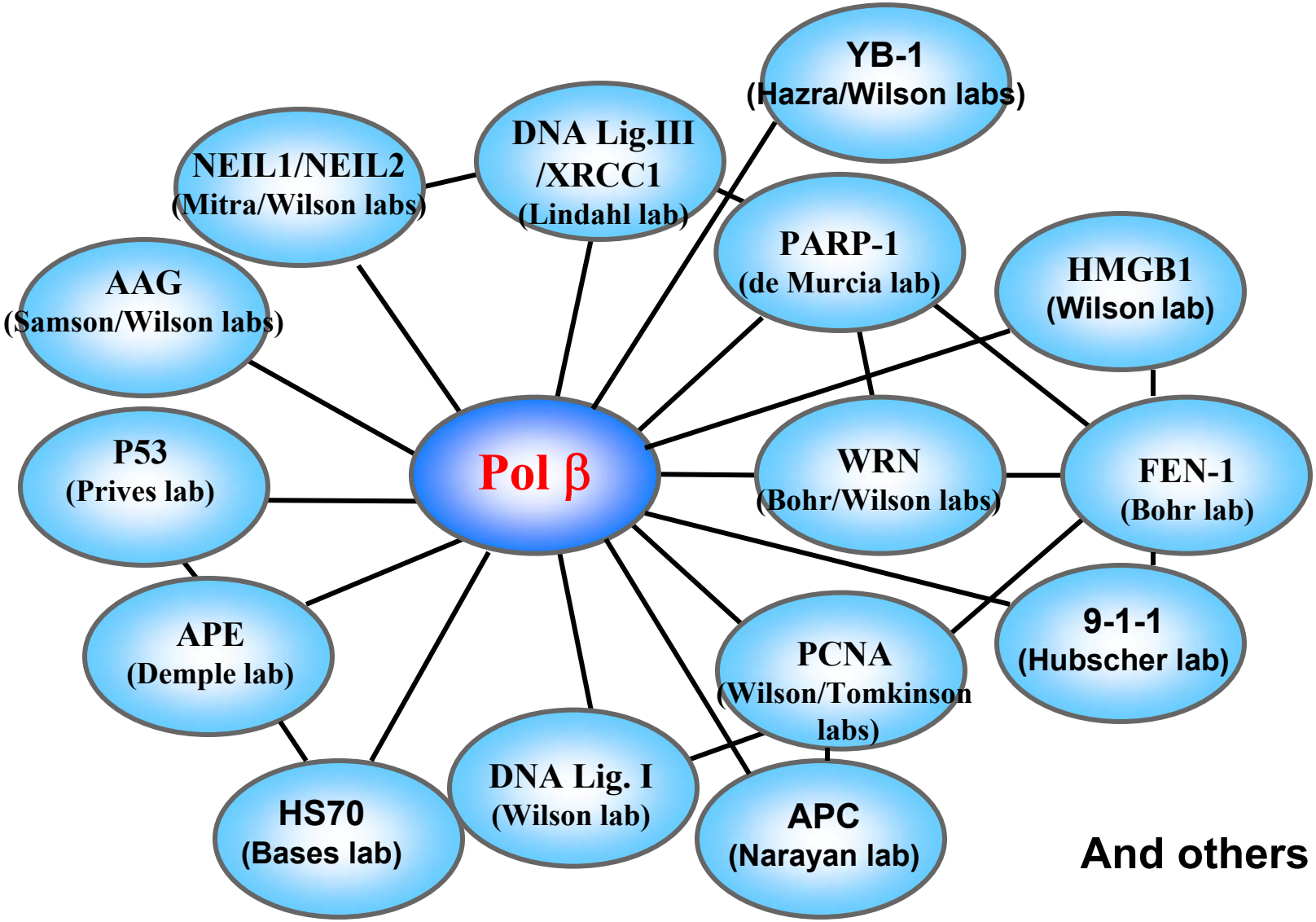
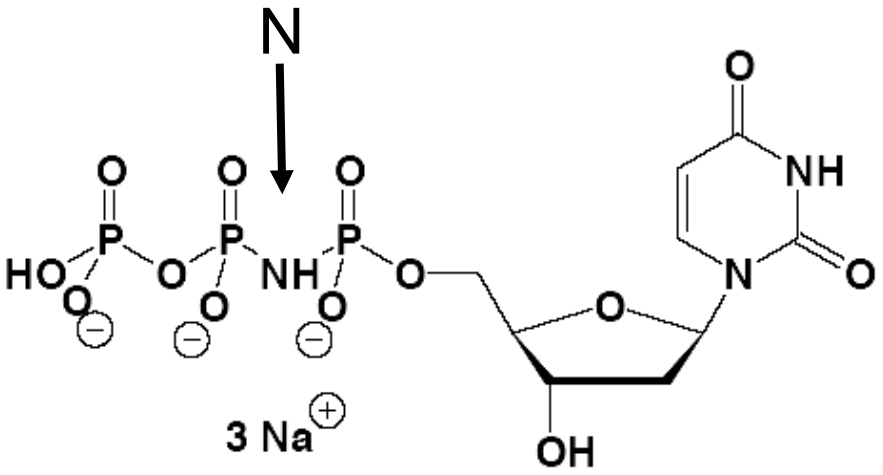
Summary of Pol ß Interactions with BER Factors (most were first identified by immunoprecipitation)



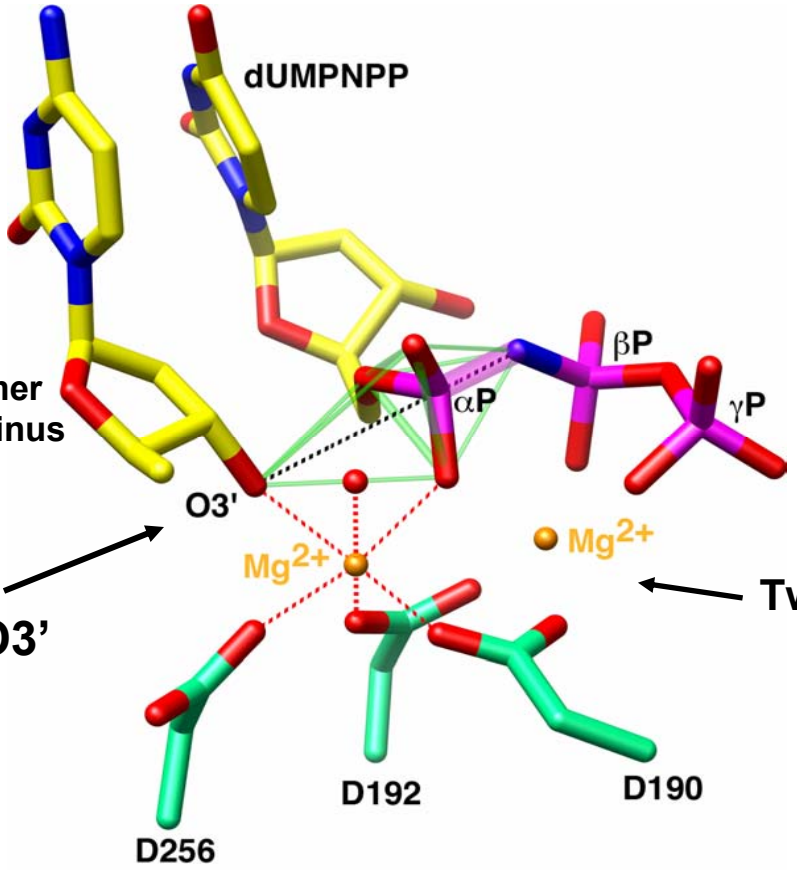
Approach

Ternary complex structure with non-hydrolyzable dUTP analog, dUMPNPP, as incoming nucleotide opposite template base dA (complex has 2 Mg ions and 3'OH of the primer).



2'-deoxyuridine-5'-[(α,β)-imido]triphoshphate (dUMPNPP)

Significance of Structure with Primer O3' intact: Both Metals in Position to Stabilize Penta-coordinated Transition State, Enabling Ouantum Mechanical Calculations of Transition State Development

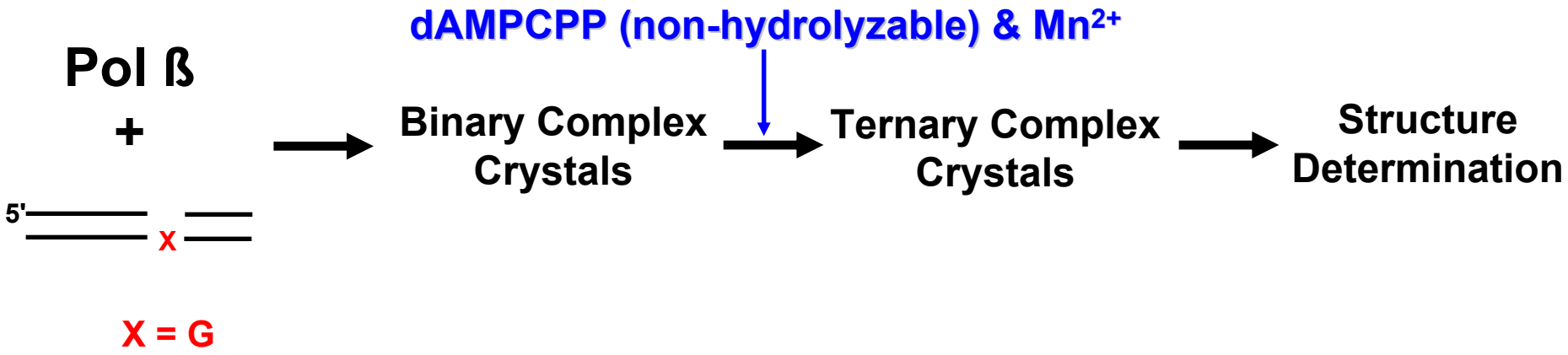


In position for "in-line attack" by O3'

Two Metals

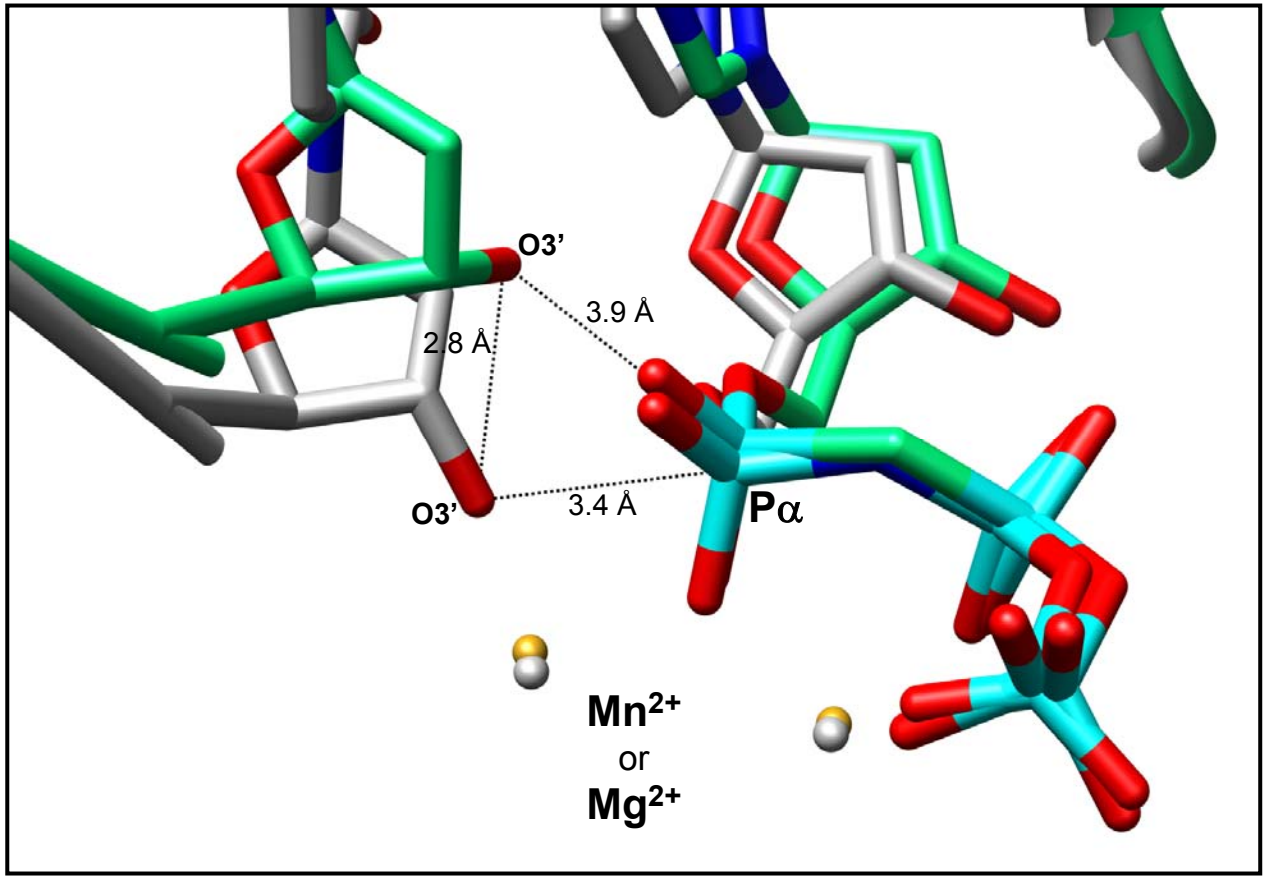
Batra et al., Structure, 14:757-766, 2006

Ternary Complex Structure with Mismatched Incoming Nucleotide



Batra et al. (in preparation)

dA—dUMPNPP (Right) **dG—dAMPCPP (Wrong)**



Polymerase Active Site

Batra et al. (in preparation)

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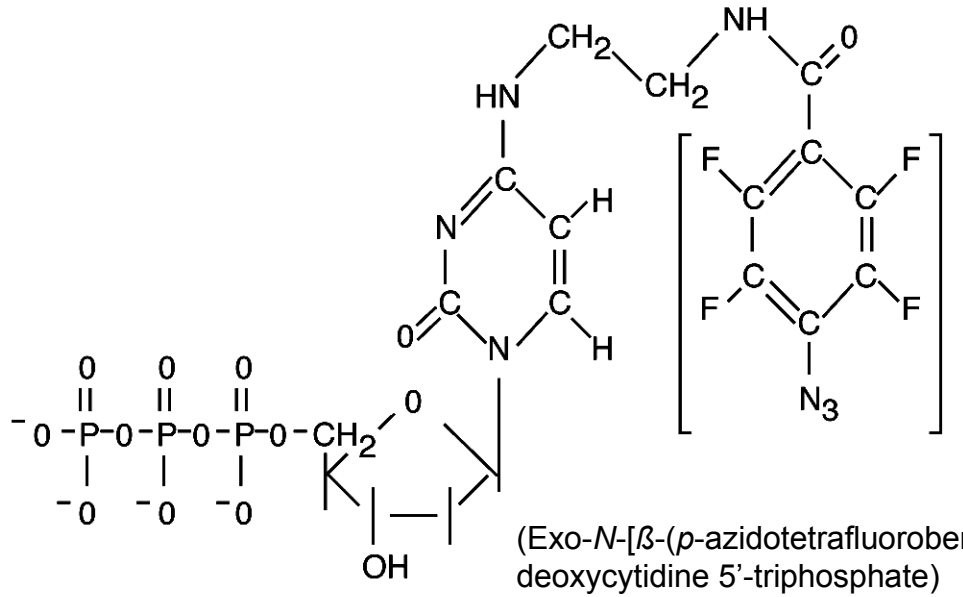
Structure of the Photoreactive dCTP Analogue (FAB-dCTP) and Oligonucleotide DNA

A



(Exo-*N*-[*ß*-(*p*-azidotetrafluorobenzamido)-ethyl] deoxycytidine 5'-triphosphate)

[d$\overset{*}{C}$TP]=FAB-dCTP

B

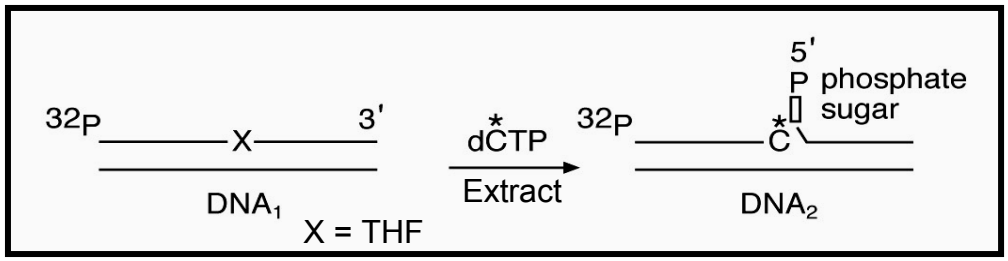
X = THF

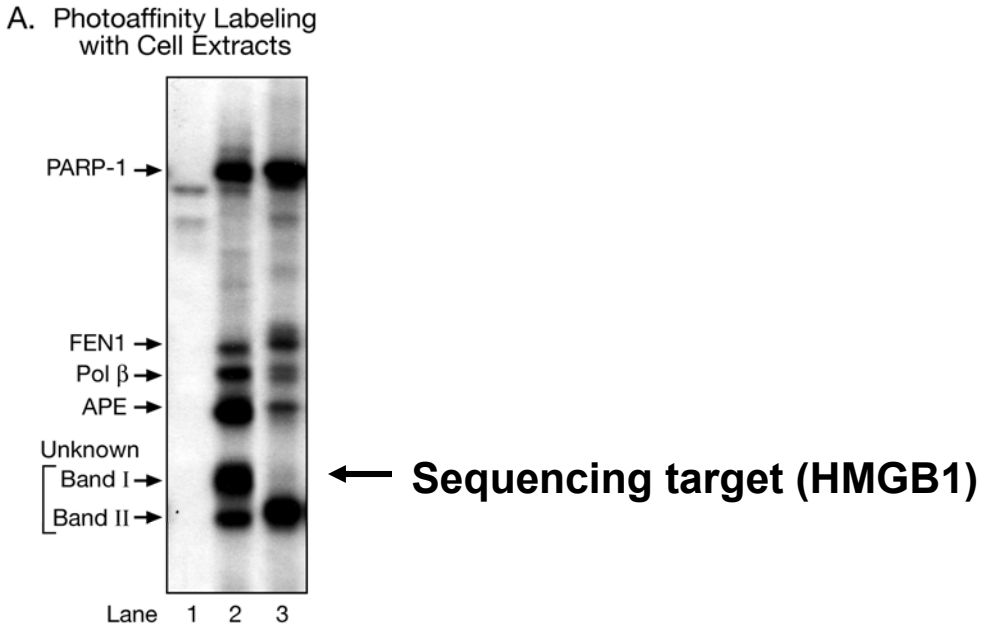
```
5'-[32P]-CTG CAG CTG ATG CGC XGT ACG GAT CCC CGG GTA C-3'
      3'-GAC GTC GAC TAC GCG GCA TGC CTA GGG GCC CAT G-5'
```

DNA_1

Olga Lavrik & associates

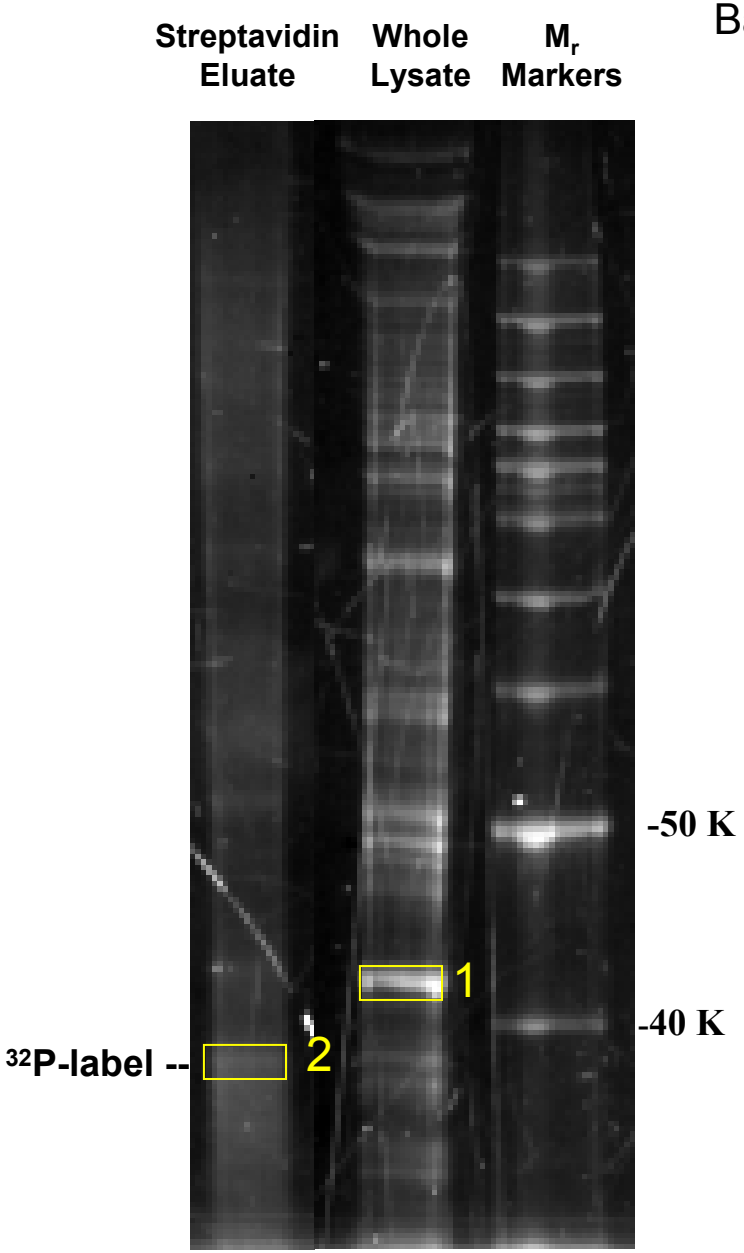
Photoaffinity Labeling of Proteins in Extracts
(identification of HMGB1 as "Unknown Band I)





1: Extract without dC*TP (control)
2: Extract from Pol ß +/+ MEF
3: Bovine testis nuclear extract (HMGB1 negative)

HMGB1 identified using MALDI/MS and MALDI/MS/MS



Band #	Protein	M_r	Score
1	Actin	41609	226
2	**HMGB1**	24892	102

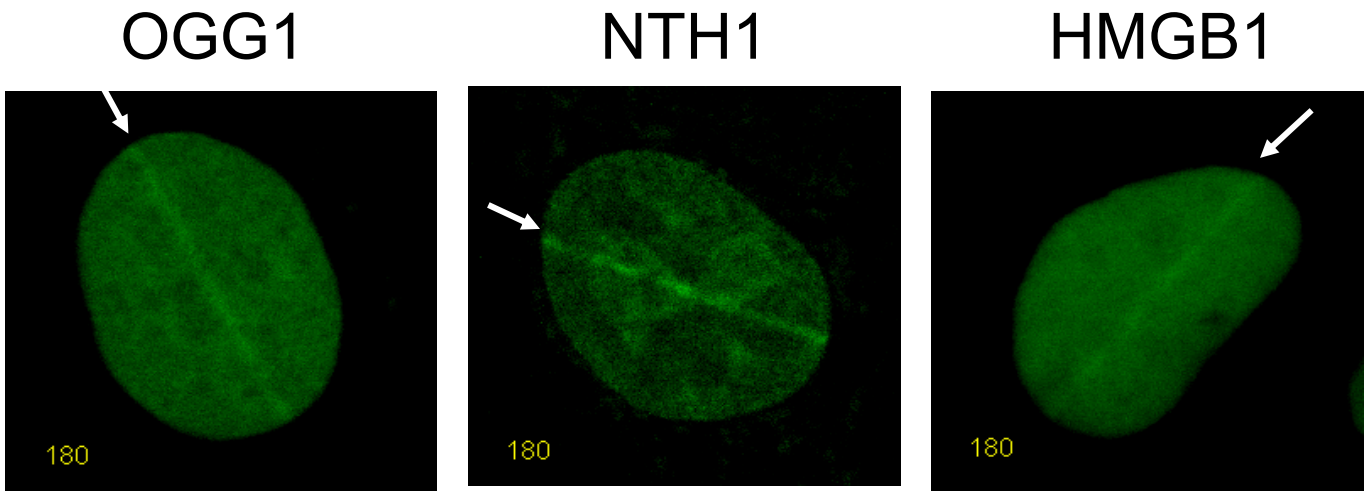
Sequence Coverage: 27%
Red: Matched peptides shown
Underlined: MS/MS

1 MGKGDPKKPR GKMSSYAFFV QTCREEHKKK
31 **HPDASVNFSE FSK**KCSERWK TMSAKEKGKF
61 EDMAKADKAR YEREMK**TYIP PKGETKKKFK**
91 **DPNAPK**RPPS AFFLFCSEYR PK**IKGEHPGL**
121 **SIGDVAKK**LG EMWNNTAADD KHPYEKKAAK
151 LKEK**YEKDIA AYR**AKGKPDA AKKGVVKAEK
181 SKKKKEEEED EEDEEDEEEE EDEEDEEEEE
211 DDDDE

(by Leesa Deterding & Ken Tomer)

Accumulation of GFP-tagged human proteins following laser-induced DNA damage

(by Akira Yasui and associates)

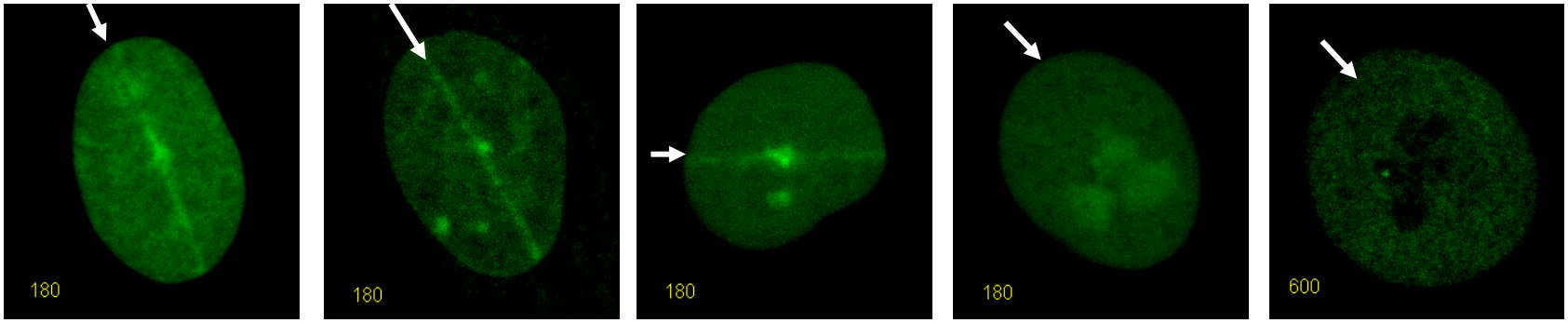


Prasad et al. (In Press)

Accumulation of GFP-tagged human proteins at DNA damage sites after laser irradiation in the presence of a photo-sensitizer

(by Akira Yasui and associates)

OGG1 NTH1 HMGB1 KU70 RAD52



10 scans in the presence of a photo-sensitizer

Photo-sensitizer: 8-MOP (methoxypsoralen)

Prasad et al. (In Press)

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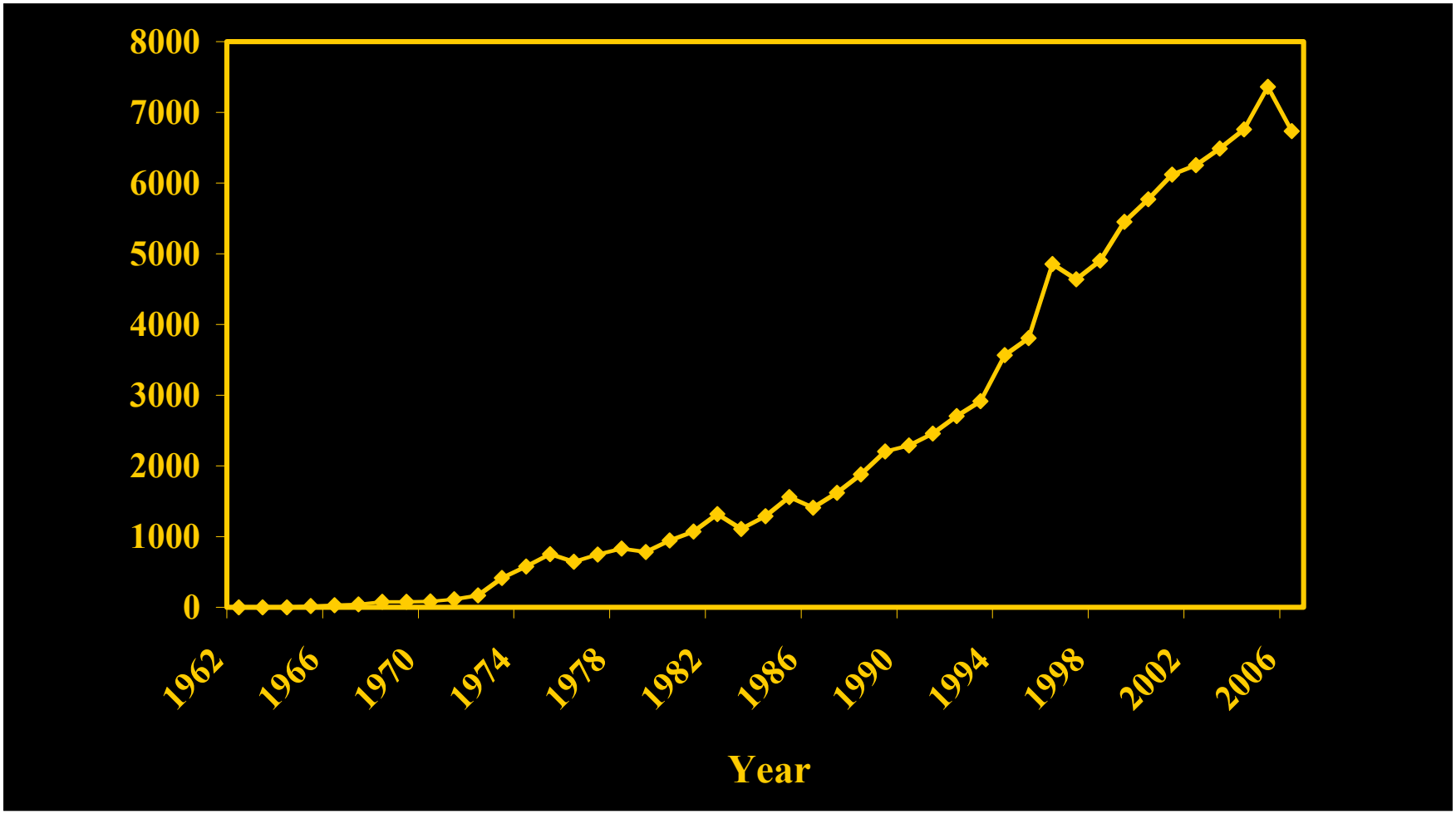
ACKNOWLEDGEMENTS

- Current associates
- Former associates
- Mentors
- Friends & Colleagues
- Competitors
- Funding (NIH & foundations)
- Current collaborators
- Former collaborators
- Service cores
- Commercial sources
- Editors & journals

DNA Repair Publications



SCOPUS searched the following terms:
TITLE-ABS-KEY (dna repair OR dna damage OR dna adducts OR dna lesions OR nucleotide excision repair OR base excision repair OR mismatch repair). Total = 105,433

(Ben Van Houten)