16

Viral DNA Polymerases

Donald M. Coen
Department of Biological Chemistry and Molecular Pharmacology
Harvard Medical School
Boston, Massachusetts 02115

Because many viruses are readily amenable to molecular, genetic, and biochemical analyses, and because the replication of certain viruses has been the target of antiviral drug development, a wealth of information has accumulated regarding the structure, functions, properties, and regulation of eukaryotic viral DNA polymerases. Viral DNA polymerases have interesting similarities with eukaryotic cellular DNA polymerases (Wang, this volume) and fascinating differences, both of which shed light on mechanisms of polymerase function.

With the exception of the papovaviruses and parvoviruses, which utilize cellular DNA polymerases for their replication, all known DNA viruses that infect animal cells encode their own DNA polymerases. In each case, the DNA polymerase is essential for the replication of the virus. Why have these viruses evolved to encode and require their own DNA polymerases? (In this chapter, only DNA polymerases that strictly utilize DNA templates are considered. Information about the reverse transcriptases encoded by retroviruses or the polymerases encoded by hepadnaviruses can be found in Skalka and Goff [1993] and in Seeger and Mason [this volume].) For poxviruses, which replicate in the cytoplasm (Traktman, this volume), the answer may be that the viral genome does not gain access to the cellular polymerases. For other viruses, the answer may be that their normal life cycles entail the productive infection of nondividing cells that do not express sufficient cellular polymerase.

Despite the fact that these viruses have each evolved a unique DNA polymerase that cannot be replaced by cellular polymerases, the viral enzymes share considerable sequence homology with eukaryotic DNA polymerases α, δ, and ε as well as *Escherichia coli* DNA polymerase II and the DNA polymerases of certain bacteriophage such as T4 and $\phi 29$ (Wong et al. 1988; Ito and Brathwaite 1991). Moreover, based on sequence alignments among a wide variety of DNA and RNA polymerases and the crystal structures of the Klenow fragment of *E. coli* DNA

DNA Replication in Eukaryotic Cells
 0-87969-459-9/96 $5 + .00

polymerase I, rat DNA polymerase β, human immunodeficiency virus reverse transcriptase, and T7 RNA polymerase, the case has been made that all polymerases share common structures and sequence motifs with specific functions, as discussed by Wang (this volume). Studies of viral polymerases have been helpful in understanding the functions of these motifs, as discussed in this chapter.

This chapter summarizes information on the functions, enzymology, pharmacology, genetics, protein-protein interactions, and regulation (often posttranscriptional) of selected viral DNA polymerases. The subunit composition and functions of several viral polymerases are presented in Table 1. For each polymerase, the holoenzyme is the form found in the virus-infected cell. However, the enzymes are often readily prepared via the use of heterologous expression systems such as recombinant baculovirus-infected cells, permitting the overexpression, purification, and analysis of the holoenzyme or each subunit.

For those viral DNA polymerases that have been most intensively studied, primer-template preferences, relevant inhibitors, and phenotypes of interesting mutants are presented in additional tables. For different polymerases, different properties are emphasized. The first group of polymerases to be discussed are those of herpesviruses. The focus is on the prototype herpes simplex virus (HSV) enzyme, which has been the object of more study than other viral polymerases. This is partly due to its being an excellent target for (profitable) antiviral drugs and to its being amenable to biochemical and genetic analysis, often with the aid of mutants isolated for resistance to antiviral drugs. The poxvirus DNA polymerases are represented by the vaccinia virus enzyme, whose features are outlined by Traktman (this volume). Further details, particularly regarding its genetics, are presented here. The third group of enzymes to be covered are those of adenoviruses (mainly adenovirus types 2 and 5). Much of the relevant information about adenovirus DNA polymerases can be found in Hay (this volume); thus, the presentation here is limited to aspects of its enzymology, genetics, and functions. Finally, aspects of the regulation of viral polymerases, in which there are interesting similarities among diverse systems, are summarized.

HSV DNA POLYMERASE

Herpesviruses

There are a multitude of herpesviruses that infect fish, birds, and mammals. Among the seven or more human herpesviruses are HSV (types 1 and 2), varicella zoster virus (VZV), human cytomegalovirus (CMV),

Table 1 Subunits of viral DNA polymerases

Virus	Catalytic subunit (names[a]; mass[b]; functions[c])	Other subunits (names; mass; functions)	Composition
HSV	UL30, pol; 137 kD; polymerase 3′→5′ Exo, RNase H	UL42, $65K_{DBP}$; 52 kD; DNA binding, processivity	pol:UL42, 1:1
CMV	UL54, pol; 137 kD; polymerase 3′→5′ Exo[d]	UL44, ICP36, p52; 46 kD; DNA binding, processivity[e]	unknown
EBV	BALF5, pol; 113 kD; polymerase, 3′→5′ Exo	BMRF1, EA-D; 43 kD; DNA binding, processivity[e]	pol:BMRF1, 1:1[f]
Adeno	E2B, pol; 135 kD; polymerase 3′→5′ Exo	pTP; 80 kD; protein primer, nuclear transport, origin-recognition	pol:pTP, 1:1
Vaccinia	E9, pol; 116 kD; polymerase 3′→5′ Exo	none known	pol monomer
Baculo	pol; 114 kD; polymerase 3′→5′ Exo[d]	none known	unknown
ASFV	G1207R, pol; 142 kD; polymerase	none known	unknown

[a]For each polypeptide, the first name listed is the name of the gene, usually the name of the open reading frame in the DNA sequence (HSV, CMV, EBV, vaccinia, ASFV). Most investigators refer to the catalytic subunits also as Pol or pol.

[b]Masses are those predicted from the DNA sequences.

[c]For catalytic subunits, only intrinsic enzymatic activities are listed. For other subunits, activities imparted on the catalytic subunit are included as well as intrinsic binding activities. Each subunit, of course, also interacts with the others.

[d]The exonuclease activity has not yet been shown rigorously to be intrinsic.

[e]Processivity has not yet been shown rigorously by template challenge.

[f]T. Tsurumi (pers. comm.).

and Epstein-Barr virus (EBV). Infections with these viruses are common, and because of their propensity to establish lifelong latent infections, these viruses often recur to cause disease. In most immunocompetent adults, herpesvirus diseases are not life-threatening but can be at least temporarily debilitating, painful, and/or emotionally troublesome. In immunocompromised adults and neonates, herpesviruses can cause severe disease and death. These clinical properties have encouraged pharmaceutical houses to develop drugs active against herpesviruses, especially HSV.

The features of herpesvirus DNA replication are reviewed by Challberg (this volume) and Yates (this volume). Germane to this chapter is that all herpesviruses encode a DNA polymerase. In each case examined thus far, the catalytic activity resides in a large subunit (110–140 kD), and there is a smaller subunit that binds DNA and stimulates polymerase activity (Table 1).

Enzymology and Pharmacology

HSV DNA polymerase consists of a catalytic subunit (pol) and a smaller subunit, UL42 (Table 1). Distinctive enzymological features of the holoenzyme and of the catalytic subunit, which has been overexpressed by itself using baculovirus expression vectors (Hernandez and Lehman 1990; Marcy et al. 1990b), are presented in Table 2. HSV DNA polymerase is able to utilize a variety of primer templates, notably poly(dC)oligo(dG), and is able to extend RNA primers. It is distinguished from most cellular polymerases by its stimulation by relatively high concentrations of salt, its relatively low K_m values for dNTPs, and its sensitivity to a variety of inhibitors. This sensitivity contributes to the success of several antiviral drugs, especially acyclovir. Acyclovir, which is a guanine base attached to an acyclic sugar moiety, is converted to the monophosphate mainly via an HSV-encoded thymidine kinase. The triphosphate, formed from the monophosphate via the action of cellular enzymes, is a much more potent inhibitor of the viral DNA polymerase than it is of cellular polymerases (Martin et al. 1994). The mechanism of inhibition entails three steps (Reardon and Spector 1989): (1) binding, which can be competed by dGTP; (2) incorporation into the growing DNA chain, leading to chain termination (acyclovir lacks a 3′ hydroxyl); (3) very potent inhibition by triphosphates complementary to the next position in the template, which prevents dissociation of the enzyme from the primer template.

Table 2 Properties of herpesvirus DNA polymerases

Form of enzyme	Salt and other optima for polymerase activity[a]	K_m for dNTPs[a]	Preferred primer templates[b]	Selected inhibitors
HSV holoenzyme (pol-UL42)	NaCl, KCl: 200–250 mM $(NH_4)_2SO_4$: 100–150 mM	0.05–0.5 μM	poly(dA)oligo(dT) poly(dT)oligo(dA)	acyclovir triphosphate phosphonoformic acid aphidicolin peptide corresponding to carboxy-terminal 36 residues of pol
HSV catalytic subunit (pol) only	less established; one report indicates lower salt concentrations are optimal (Hart and Boehme 1992)	similar	activated DNA poly(dT)oligo(rA) poly(dC)oligo(dG)	sensitive to all of the above except peptide
CMV holoenzyme (pol-UL44)	NaCl, KCl: 100–150 mM $(NH_4)_2SO_4$: 60–100 mM	0.5 μM	activated DNA poly(dA)oligo(dT) primed ss bacteriophage DNA	acyclovir triphosphate phosphonoformic acid aphidicolin
CMV catalytic subunit (pol) only	similar	less established	poly(dC)oligo(dG)	similar
EBV holoenzyme (pol-BMRF1)	$(NH_4)_2SO_4$: 100 mM	0.5 μM	poly(dA)oligo(dT) poly(dC)oligo(dG) primed ss bacteriophage DNA	acyclovir triphosphate phosphonoacetic acid aphidicolin
EBV catalytic subunit (pol) only	$(NH_4)_2SO_4$: 0 mM		activated DNA poly(dT)oligo(rA)	similar

Selected references: HSV, Weissbach et al. (1973); Derse and Cheng (1981); Derse et al. (1982); Frank et al. (1984); Reardon and Spector (1989); Gottleib et al. (1990); Hart and Boehme (1992); Hamatake et al. (1993); Digard et al. (1995). CMV, Nishiyama et al. (1983); Mar et al. (1985); Ertl and Powell (1992); Weiland et al. (1994). EBV, Chiou and Cheng (1985); Tsurumi et al. (1993a,b).

[a]Using activated DNA as primer template. Ranges reflect differences in published values and among different dNTPs. Different values are obtained with other primer templates.

[b]Those listed for catalytic subunit only are utilized with similar efficiency by the holoenzyme. Those only listed for holoenzyme are generally utilized much more efficiently by the holoenzyme than the catalytic subunit.

HSV DNA polymerase contains an intrinsic 3′→5′ exonuclease activity and an intrinsic RNase H activity (Crute and Lehman 1989; Marcy et al. 1990b; Weisshart et al. 1994). There is controversy regarding whether the RNase H functions as a 5′→3′ exonuclease (Crute and Lehman 1989) or whether it is instead a manifestation of the 3′→5′ exonuclease activity (Knopf and Weisshart 1990; Knopf and Strick 1993).

The catalytic subunit alone is much less active than the holoenzyme for polymerization on certain primer templates (Table 2), including poly(dA)oligo(dT) and singly primed M13 DNA (Gottleib et al. 1990; Hamatake et al. 1993). This is due to the ability of the small subunit, UL42, to stimulate processivity (Gottleib et al. 1990; Hernandez and Lehman 1990), which correlates with increased affinity for DNA in a primer-template configuration (Gottleib and Challberg 1994; B.D. Song; D. Herschlag; I.R. Lehman; all pers. comm.; K. Weisshart and D.M. Coen, unpubl.). UL42 is an unusual processivity factor in that it has intrinsic affinity for double-stranded DNA, which is required for processivity (Chow and Coen 1995).

UL42 also binds specifically to the catalytic subunit, and this binding too is required for processivity (Digard et al. 1993a,b). It is likely that these properties are shared by other small herpesvirus polymerase subunits like EBV BMRF1 and CMV UL44, which also have intrinsic DNA-binding activity and stimulate their cognate catalytic subunits (Tables 1 and 2). The current working model then is that UL42 simultaneously binds pol and DNA and thereby increases affinity for primer template. How this can occur without braking elongation (a problem solved by processivity factors such as proliferating cell nuclear antigen by wrapping around DNA without binding, thereby creating a "sliding clamp") is an area of active investigation.

The interaction of HSV pol and UL42 is not only required for processivity in vitro; it is also required for viral replication in vivo (Digard et al. 1993a,b). Thus, this interaction could be the target for antiviral drugs. Residues crucial for the interaction lie at the extreme carboxyl terminus of pol (Digard et al. 1993a; Stow 1993; Tenney et al. 1993b). Accordingly, certain peptides corresponding to the carboxyl terminus of pol block UL42-dependent DNA synthesis catalyzed by pol at concentrations that had no effect on synthesis by pol alone or by heterologous polymerases (Table 2) (Marsden et al. 1994; Digard et al. 1995). Thus, these peptides represent a new class of polymerase inhibitors that act by blocking accessory-subunit-dependent synthesis. They or their structures may form the basis for the synthesis of antiviral drugs.

Genetics: Drug Resistance and Conserved Regions

HSV is an excellent genetic system, and this has been advantageous in studies of HSV DNA polymerase. Many genetic studies have focused on mutations in the *pol* gene that alter the sensitivity of the virus to drugs. Such mutations alter the catalytic subunit so that it is functional, yet exhibits altered specificity. As the drugs mimic and/or compete for binding with natural polymerase substrates, the mutations can be expected to alter amino acids that are within or impinge on the binding site for the substrate. Clusters of such mutations should identify regions that are directly or indirectly involved in binding the substrates.

In the HSV system, this approach has mainly identified amino acids in six regions that are conserved among many viral and cellular polymerases of the α-like family (also known as family B; see Wang, this volume). These regions are designated as I, II, III, V, and VII, which are found in almost all α-like polymerases, and δ-region C (Zhang et al. 1991), which is found in cellular DNA polymerases δ and ε, but not α (Fig. 1, Table 3). Thus, these regions are presumably conserved for their role in polymerase catalysis. Because regions II and III contain the greatest clustering of such mutations (Table 3), it was proposed that these segments would be likely to directly interact with substrates and drugs (Gibbs et al. 1988). As reviewed by Wang (this volume), there is consid-

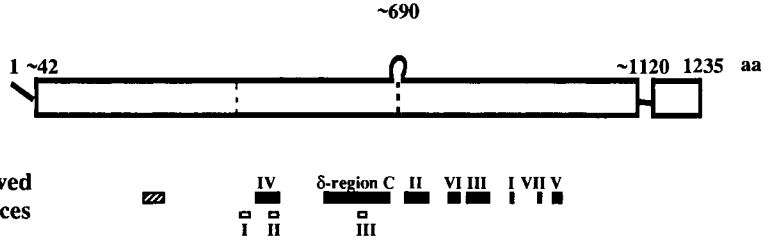

Figure 1 Locations of conserved regions and structural domains on viral DNA polymerases using the HSV DNA polymerase catalytic subunit as an example. The 1235-amino acid (aa) HSV pol polypeptide is represented as lines, indicating less-ordered regions susceptible to proteolytic digestion, with the residue numbers indicating the sites of cleavage (Weisshart et al. 1994) and boxes representing structural domains. Dashed lines indicate sites of proteolytic digestion that do not separate structural domains. Below are the locations of conserved regions I–VII shared among α-like polymerases (Wong et al. 1988; Hwang et al. 1992) and δ-region C (Zhang et al. 1991) as filled boxes; ExoI, II, and III (Bernad et al. 1989) as open boxes; and a segment with some homology to RNase H enzymes and certain α-like polymerases (Reha-Krantz 1990) as a hatched box.

Table 3 Altered drug sensitivity mutations affecting conserved regions of viral DNA polymerases

	δ-Region C (includes ExoIII and region A)	Region II
Consensus	XXXXLSSYKLNXVA..XXXEXARXXGIPhXRXhXXGQQ	YNGGXXhXPXXG..VhhDhNSLYPSIMX
	A	
HSV wild type	531	696
	DKIKLSSYKLNAVA..ELSAVARLAGINITRTIYDGQQ	YQGARVLDPTSG. .VVFDFASLYPSIIQ
HSV mutations	N K V	H G V N*
	D	
CMV mutations	I I	
VZV mutations	K	Y T
Vaccinia mutations	V	
	T	
	(wild type A)	

	Region III	Region I
Consensus	XQhhhKLXXNShYGXXGXXX..XXhhhXXhXXXGRX	hXhIYGDTDSIFV
HSV wild type	806	880
	QQAAIKVVCNSVYGFTGVQH..CLNVAATVTTIGRE	MRIIYGDTDSIFV
HSV mutations	M S* C M SS	*A*CM
	C	Y
VZV mutations	S	L
Vaccinia mutations	M	

	Region VII	Region V
Consensus	XKKXYhhX	KXXXKGXXXXRXXXX
HSV wild type	937	949
	AKKKYIGV	KMLIKGVDLVRKNNC
HSV mutations	H	K
CMV mutations		G

	ExoI	ExoII
Consensus	hhXFDIEXXX	hXGXNXXXFDXXXIhXXL
CMV wild type	297	404
	CLSFDIECMS	VTGYNINSFDLKYILTRL
CMV mutations	N	V
Vaccinia mutations	S	G
	(wt F)	

For each conserved region, the first line provides a consensus sequence showing residues shared among many family members in single-letter code with X indicating no clear consensus and h indicating a hydrophobic residue. In one case, where two amino acids predominate, both are indicated. The second line provides, in most cases, the wild-type HSV sequence and for ExoI and ExoII, the CMV sequence with the number of the first residue shown. Portions of the sequence omitted are indicated as "..". Below these are indicated residues found in *pol* mutations from viruses exhibiting altered drug sensitivity. In a few cases, it has not yet been demonstrated that the mutation observed actually causes the altered drug sensitivity phenotypes. References: HSV, Larder et al. (1987); Gibbs et al. (1988 and references therein); Hall et al. (1989); Hwang et al. (1992); Wang et al. (1992); Chiou et al. (1995). VZV, K. Biron (pers. comm.). CMV, Lurain et al. (1992); Sullivan et al. (1993); Lurain et al. (1994). Vaccinia, Earl et al. (1986); Traktman et al. (1989); Defilippes (1989); Taddie and Traktman (1991); Taddie and Traktman (1993). The asterisk (*) refers to mutations that affect drug sensitivity of HSV pol in vitro but which have not been tested in the authentic context of the virus genome. Thus, although the N in region II and the S in region III that are asterisked have been tested in the context of the virus genome, substitutions to A, D, E, H, K, Q, and T at the former position and A, D, E, G, L, Q, V, and Y (Matthews et al. 1993) at the latter position have not, but do affect drug sensitivity of HSV pol in vitro. The S to A substitution that is asterisked and italicized in region I was only tested in vitro (Dorksy and Plourde 1993).

erable support for this proposal based on structural studies of other polymerases that appear to contain homologs of these regions and from enzymological studies of mutant DNA polymerase-α and other α-like polymerases. Region I appears to be involved in catalysis, rather than affinity for substrates, so its involvement in substrate and drug recognition may be more indirect. Aside from drug-resistance mutations, certain mutations in this region can inactivate polymerase activity in vitro (Dorsky and Crumpacker 1990) and viral replication in vivo (Marcy et al. 1990a). The precise roles of regions V and VII are poorly understood at present.

The first mutations in δ-region C were suggested to identify a 60-residue segment (then known as region A) that was specific to viruses which are sensitive to antiviral drugs (Gibbs et al. 1988). Subsequently, this region was found to comprise a much longer segment (>100 residues in HSV) that is also conserved among cellular DNA polymerases δ and ε (Zhang et al. 1991). Interestingly, a drug-resistance mutation isolated by a suppressor approach maps to the amino-terminal portion of this segment (Wang et al. 1992). The mapping of the mutations suggests that δ-region C could also form part of the dNTP-binding site, even though it also contains the ExoIII segment, one of three regions (ExoI, II, and III, see Fig. 1) that are conserved among the active sites of 3′→5′ exonucleases (Bernad et al. 1989). These and other data (see below) argue for interdependence between portions of the enzyme involved in polymerization and exonuclease activities.

Of all the *pol* mutations that confer altered drug sensitivity to HSV, only two do not map to regions conserved among many α-like polymerases. One of these lies adjacent to region III and alters a proline and, thus, could conceivably act by shifting the orientation of region III (Gibbs et al. 1988). The other, isolated for suppression of phosphonoacetic acid (PAA)-hypersensitivity (Wang et al. 1992), lies in a region of sequence similarity shared among a few α-like polymerases and certain RNase Hs (Fig. 1) (Reha-Krantz 1990). The significance of this has not been established.

Several of the drug-resistant mutants exhibit an antimutator phenotype (Hall et al. 1984, 1985; Wang et al. 1992; Hwang and Chen 1995). For at least one mutant, PAA^{r}5, this appears to result from decreased misincorporation of incorrect bases during polymerization associated with higher K_m values for dNTPs rather than increased editing by the 3′→5′ exonuclease (Hall et al. 1985). At the sequence level, the wild-type polymerase mediates transition and frameshift mutations at about a 1:1 ratio in an HSV gene. Interestingly, the PAA^{r}5 enzyme ex-

hibits an altered spectrum of mutations, being especially impaired in mediating transition mutations (Hwang and Chen 1995). The mechanisms underlying the antimutator phenotype for this and other mutants deserve further study.

Mapping of Activities

Numerous mutations have been constructed in the *pol* and *UL42* genes and tested for their effects on enzyme function in vitro and virus replication in vivo. Deletion analysis indicates that the catalytic subunit can suffer deletions as large as 227 residues at the amino terminus and 58 residues at the carboxyl terminus without loss of polymerase activity in vitro (Dorsky and Crumpacker 1988; Haffey et al. 1990), although the latter deletion and other mutations at the carboxyl terminus impair association with UL42 and its stimulation of long-chain DNA synthesis as well as viral replication (Digard et al. 1993a; Stow 1993; Tenney et al. 1993b; Marsden et al. 1994). Current data support the concept of an approximately 36-residue subdomain at the extreme carboxyl terminus of HSV pol that interacts with UL42 (Digard et al. 1995). This subdomain appears to be part of an approximately 12-kD carboxy-terminal structural domain identified by limited proteolysis (Fig. 1). This domain also has DNA-binding activity and is required for polymerase activity (Digard et al. 1993a; Weisshart et al. 1994; W.R. Bebrin et al., unpubl.). Limited proteolysis has also shown that the amino-terminal half of the pol molecule is sufficient for its 3′→5′ exonuclease and RNase H activities (Weisshart et al. 1994). However, deletions and single and clustered point mutations in many locations in the *pol* gene, including the amino-terminal half, ablate polymerase activity (Dorsky and Crumpacker 1988, 1990; Haffey et al. 1990; Gibbs et al. 1991; W.R. Bebrin et al., unpubl.). Interestingly, mutations within conserved region IV in the ExoII segment inactivate polymerization activity, yet the protein retains certain other activities (Gibbs et al. 1991). These data, taken together with the drug-resistance data cited above, tend *not* to support a model in which polymerase activity resides on a structurally and functionally independent polymerase domain à la *E. coli* DNA polymerase I (Derbyshire et al. 1993). Rather, it appears that HSV polymerase activity requires contributions from other domains and/or active sites. Related results in other viral systems are discussed below.

Limited proteolysis has also mapped sites of conformational changes that occur in pol upon DNA binding (Weisshart et al. 1993). One site is in or near the "hinge" between the 12-kD carboxy-terminal domain and

the rest of the molecule (Fig. 1). The other is in the vicinity of δ-region C and region II (Fig. 1).

The UL42 subunit has been studied less extensively than pol. Its most surprising feature is an approximately 175-residue carboxy-terminal tail with no apparent function in vitro or in vivo (Digard et al. 1993b; Gao et al. 1993; Hamatake et al. 1993; Monahan et al. 1993; Tenney et al. 1993a). However, most mutations in the remaining approximately 315 amino acids either inactivate all three UL42 activities identified (DNA binding, pol binding, processivity) or have little effect on any. The important exceptions are a linker insertion mutation at codon 160, which inactivates pol binding without apparent effect on DNA binding, and linker insertions at codons 203 and 206, which diminish DNA binding without apparent effect on pol binding. Each of these mutations severely impairs the ability of UL42 to stimulate pol processivity and to support viral replication (Digard et al. 1993b; Chow and Coen 1995). Whether these mutations identify short contiguous pol- or DNA-binding sites, respectively, is not clear.

Interactions with Other Proteins

It would be surprising if there were no interactions of either pol or UL42 with other HSV replication proteins other than with each other (see Challberg, this volume). However, as yet, little direct evidence has been published for such interactions.

OTHER HERPESVIRUS DNA POLYMERASES

Enzymology and Pharmacology

The other herpesvirus DNA polymerases have not been studied as extensively as HSV DNA polymerase, but several have provided interesting insights. There is reason to suspect complications in the stoichiometries of the subunits of these enzymes. Although the EBV enzyme is evidently a 1:1 heterodimer (T. Tsurumi, pers. comm.), the EBV catalytic subunit is maximally stimulated by its accessory protein, BMRF1, at a ratio of two BMRF1 molecules to each EBV pol (Tsurumi et al. 1993a). The murine cytomegalovirus homolog of the CMV accessory protein forms disulfide-linked homodimers and higher-order forms (Loh et al. 1994). The CMV and EBV holoenzymes exhibit many of the properties of the HSV enzyme (Table 2). However, the catalytic subunits

alone differ substantially in their salt optima and preferred primer templates, and these parameters strongly influence the response of the catalytic subunits to their cognate accessory proteins (Table 2 and references therein). Thus, it is not yet clear to what extent these other herpesvirus accessory proteins function similarly to HSV UL42.

Like its HSV counterpart, the EBV pol contains an intrinsic 3′→5′ exonuclease activity (Tsurumi et al. 1993b). Ribonucleoside monophosphates inhibit this activity, but not polymerase (Tsurumi 1992; Tsurumi et al. 1994), unlike the HSV holoenzyme (Frank and Cheng 1986). Interestingly, the BMRF1 accessory subunit enhances the dsDNA nuclease activity of EBV pol, but not its activity on ssDNA (Tsurumi et al. 1994).

Genetics: Drug Resistance and Conserved Regions

A number of drug-resistant VZV and CMV mutants with lesions in the *pol* gene have been isolated. The five VZV mutations are very similar in location to those of HSV (Table 3). One mutant contains an alteration in region II not previously observed, and the others contain residues altered in regions I, II, and III and in δ-region C corresponding to residues altered in HSV mutants. The five CMV mutants, however, have a rather unusual spectrum of alterations (Table 3). This may be due to their selection for resistance to the nucleoside analog, ganciclovir. None of the mutations is in regions I–III. One is in region V and two are in δ-region C, bolstering the cases that these segments are involved in substrate recognition. Most surprising, two of the mutations are in highly conserved residues within the ExoI and ExoII segments. The aspartate altered in the ExoI segment is believed to be involved in metal binding and is required for exonuclease activity in other α-like polymerases (Bernad et al. 1989; Morrison et al. 1991; Simon et al. 1991). The phenylalanine altered in ExoII is adjacent to an aspartate that has similar properties to that in ExoI (Bernad et al. 1989; Simon et al. 1991). One interpretation of these results is that the mutations may affect the dNTP-binding site of CMV pol (Lurain et al. 1992). A second interpretation is that ganciclovir resistance is due to increased exonuclease activity to remove incorporated drug; however, the mutations would be expected to decrease exonuclease activity. A third possibility is based on the ability of ganciclovir triphosphate to promote chain termination despite its having a 3′ hydroxyl residue (Reid et al. 1988). Perhaps, the polymerase is less likely to stall and come off the primer template following drug in-

corporation when the exonuclease is less active, leading to internal incorporation of drug and subsequent repair or proper copying. These alternatives can be addressed by biochemical analysis of mutant polymerases.

Aside from these drug-resistant mutants, Ye and Huang (1993) have engineered point and small deletion mutations into CMV pol and assessed effects following in vitro expression. Mutations in regions I, II, III, and V severely diminished enzyme activity, as expected. A mutation in region III abolished DNA binding in a co-sedimentation assay, but whether this was a specific effect is not clear. Interestingly, mutations in regions I and II retained DNA-binding activity but did not detectably bind substrate. Further quantitative studies will be required to relate these results to the functional roles of the conserved regions.

Mapping of Activities

There have been some mutational analyses of catalytic and accessory subunits in other herpesvirus systems. Fairly small deletions of the amino terminus of EBV pol severely diminish activity in vitro (Kiehl and Dorsky 1991; Lin et al. 1991). In pseudorabies virus (PRV, a pig virus similar to HSV), deletion of the last 30 residues of the catalytic subunit results in an active polymerase that, however, is not stimulated by the PRV homolog of HSV UL42 (Berthomme et al. 1995). Remarkably, despite this functional analogy of the carboxyl terminus of PRV pol to that of HSV pol, there is little or no sequence homology, and the HSV and PRV catalytic subunits are not stimulated by the noncognate accessory proteins. This provides an interesting example of evolution of protein-protein interactions.

The accessory subunits, CMV UL44 and EBV BMRF1, have been analyzed for effects on DNA binding and pol stimulation. As was the case for HSV UL42, fairly large carboxy-terminal deletions had little or no effect (Weiland et al. 1994; Kiehl and Dorsky 1995). Most deletions within the remaining approximately 310 residues destroyed pol stimulation and DNA binding, with the exception of small deletions in BMRF1 between residues 194 and 233, which permitted DNA binding, but not stimulation. This suggests the existence of a bipartite DNA-binding region for this protein (Kiehl and Dorsky 1995); however, further work will be required to determine if given deletion mutations specifically affected DNA binding or more globally affected protein folding. As yet, mutations that specifically impair binding to the cognate pol have not been published.

VACCINIA VIRUS DNA POLYMERASE

Vaccinia virus, the descendant of the virus used by Jenner to vaccinate against smallpox, is the prototype poxvirus. The poxviruses have a number of fascinating biological features and are amenable to biochemical and genetic analysis. The vaccinia virus DNA polymerase was among the first animal virus DNA polymerases to be purified to near homogeneity (Challberg and Englund 1979a) and, unlike the other viral enzymes discussed here, is a monomer (Table 1). The protein has recently been overexpressed using an engineered vaccinia virus vector (McDonald and Traktman 1994a). Molecular genetic approaches, which can be especially rapid in the vaccinia system, have yielded many insights that have reinforced, complemented, and extended related work in herpesvirus systems.

Enzymology, Pharmacology, and Protein-protein Interactions

Selected enzymological features of the single subunit vaccinia DNA polymerase are presented in Tables 1 and 4. It contains an intrinsic 3′→5′ exonuclease (Challberg and Englund 1979a) but lacks detectable 5′→3′ exonuclease activity (McDonald and Traktman 1994a). Its polymerase activity is perhaps most distinguished by a limited repertoire of preferred primer templates, with best activity on activated DNA and poly(dC)oligo(dG); less activity on other homopolymeric DNA primer templates; poor activity on primed single-stranded bacteriophage DNA where it stalls at areas of secondary structure (Challberg and Englund 1979b); and little or no activity on RNA-primed homopolymers, consistent with the proposed mechanism of viral DNA replication (see Traktman, this volume). It is sensitive to aphidicolin, but less so than HSV DNA polymerase or DNA polymerase-α (Pedrali-Noy and Spadari

Table 4 Properties of vaccinia virus DNA polymerases

Form of enzyme	Optima	K_m for dNTPs	Primer templates	Selected inhibitors
116-kD monomer	50 mM KPO_4 pH 8–9 5 mM $MgCl_2$, but more processive at 1 mM	1–4 μM	good: activated DNA, poly(dC)oligo(dG) poor: RNA primed DNA, primed ss bacteriophage DNA	aphidicolin (modest), arabinosyl CTP

Selected references: Citarella et al. (1972); Challberg and Englund (1979a,b); Pedrali-Noy and Spadari (1980); McDonald and Traktman (1994a,b).

1980). It is also sensitive to a number of other inhibitors that are active against herpesvirus DNA polymerases, such as arabinosylcytosine (araC) triphosphate.

The enzyme is not processive as a monomer except at low ionic strength and $MgCl_2$ concentrations and lacks the strand-displacement activity required by current models for viral DNA replication (Challberg and Englund 1979b; McDonald and Traktman 1994b; see Traktman, this volume). The enzyme's activity on primed M13 DNA can be greatly stimulated by *E. coli* single-strand DNA-binding protein, which presumably is due to destabilization of secondary structure rather than specific protein-protein interactions (McDonald and Traktman 1994b). The physiological significance of these observations is not yet certain, but they have provided a starting point for purification of accessory factors from virus-infected cells (see Traktman, this volume).

Genetics

A number of studies have examined drug-resistant vaccinia virus mutants with lesions in the *pol* gene and correlated phenotype (including antimutator and mutator behaviors) with amino acid changes in conserved motifs, much as has been done with herpesviruses (see above). The results are summarized in Table 3. The mutations analyzed confer resistance or hypersensitivity to aphidicolin, PAA, and/or araC. As with CMV, the mutations have a different spectrum of locations from those observed with HSV and VZV. None of the mutations is in regions I, II, or V. One mutation is in region III. Two alter a residue in δ-region C, and three others are in a nonconserved region in vaccinia virus DNA polymerase that splits δ-region C into two halves. This further bolsters the case that δ-region C is involved in substrate recognition. Finally, as was the case with a CMV mutant, one mutant, isolated for araC-resistance, is in the ExoI segment. The three possible interpretations suggested earlier for the CMV ganciclovir-resistance mutations in ExoI and ExoII apply here as well. Taddie and Traktman (1993) tended to favor the idea that the mutation may improve the ability of the exonuclease to remove araC residues. Regardless, the mapping of the mutations favors a model of interdependence between polymerase and exonuclease sites (Taddie and Traktman 1993).

As yet, there has been little analysis of the effects of mutations on different polymerase activities in vitro. With the overexpression and ready purification of enzyme (McDonald and Traktman 1994a) and the hunt for interacting proteins, these studies may develop in the near future.

ADENOVIRUS DNA POLYMERASE

Adenoviruses infect birds and mammals. In humans, they are a cause of respiratory disease and molecular biological research. The in vitro reconstitution of adenovirus DNA replication by Challberg and Kelly (1979) led to the identification of all the factors required, including a previously unrecognized DNA polymerase (see Hay, this volume). The catalytic subunit, which also has 3′→5′ exonuclease activity, is normally found in a 1:1 heterodimer with the precursor of the terminal protein (pTP). Mature terminal protein (TP) is bound covalently to both ends of virion DNA via linkage to the 5′ phosphates. This covalent linkage is accomplished by the polymerase, which utilizes pTP as a protein primer and incorporates dCTP into a pTP-dCMP initiation complex. Whereas with herpesvirus and vaccinia virus DNA polymerases much of the emphasis has been on issues of substrate and drug recognition, with adenovirus DNA polymerase the emphasis has been on the details of its role in the replication of adenovirus DNA. These are covered thoroughly by Hay (this volume), so the coverage here is brief and focuses on other aspects.

Enzymology and Pharmacology

Aside from the origin-dependent elongation reactions using various viral and cellular proteins (covered by Hay, this volume), adenovirus DNA polymerase by itself is typically assayed in two ways (Table 5): (1) incorporation of dNTPs on conventional primer templates and (2) incorporation of dCTP into the pTP-dCMP initiation complex, typically using viral origin sequences as template. A number of features distinguish adenovirus DNA polymerase from other viral and cellular enzymes (Table 5). The enzyme is rather salt-sensitive and is stimulated by ATP (Lichy et al. 1981; Field et al. 1984), evidently without hydrolysis to ADP (Lindenbaum et al. 1986). On its own, the enzyme is resistant to aphidicolin, unlike most other members of the α-polymerase family (Enomoto et al. 1981; Lichy et al. 1981).

A remarkable aspect of adenovirus DNA polymerase is how its behavior changes depending on the presence of particular proteins and/or primer templates. Addition of adenovirus DNA-binding protein (DBP) allows the enzyme to utilize poly(dT)oligo(dA) efficiently (Field et al. 1984). This is reminiscent of the stimulation of herpesvirus catalytic subunits on certain primer templates by their accessory subunits; however, in this case, DBP is not a strongly associating subunit of the polymerase. (The presence or absence of pTP seems to have little effect on the

Table 5 Features of adenovirus DNA polymerase

Assay	Optima	K_m for dNTPs	Primer templates	Inhibitor responses[a]
Elongation	pH 7.5–8, no added salt, 3–5 mM ATP	1 μM[b]	good: activated DNA, poly(dC)oligo(dG) poor[c]: poly(dT)oligo(dA), poly(dT)oligo(rA)	sensitive to ddCTP (100 μM) resistant to aphidicolin (100 μM)
pTP-dCMP formation	same as above	4 μM[d]	Ad virion DNA (with TP) ss adenoviral DNA	resistant to both ddCTP and aphidicolin

Selected references: Lichy et al. (1981); Lichy et al. (1982); Nagata et al. (1982); Field et al. (1984); Mul and van der Vliet (1993).

[a]Elongation with all the components required for replication of full-length viral DNA in vitro is relatively sensitive to aphidicolin (IC_{50} <10 μM).

[b]Value is for elongation following formation of an initiation complex on the adenovirus origin and is in the presence of DBP (Mul and van der Vliet 1993).

[c]In the presence of DBP, these primer templates are used fairly efficiently (Field et al. 1984).

[d]Value shown is in the absence of the adenovirus DBP. With DBP, the K_m for formation of pTP-dCMP drops to 0.5 μM (Mul and van der Vliet 1993).

polymerase's catalytic properties.) The enzyme's sensitivity to inhibitors is highly plastic. The enzyme is sensitive to ddCTP on activated DNA templates or during elongation on adenoviral DNA, but it is resistant to the inhibitor during incorporation of dCTP into pTP. Perhaps most surprising is the response to aphidicolin. Despite the enzyme's inherent resistance to aphidicolin, DNA synthesis by the polymerase using complete reconstituted systems or crude extracts is relatively sensitive to the drug (Krokan et al. 1979; Longiaru et al. 1979; Nagata et al. 1983). An appealing possibility is that the other proteins alter the conformation of the polymerase to render it sensitive to aphidicolin. This scenario was invoked to explain how certain HSV mutations in the HSV-DBP gene conferred hypersensitivity to aphidicolin (Chiou et al. 1985). Alternatively, the replication activities of one of the other components in the reaction may be aphidicolin-sensitive.

A fundamental difference between adenovirus DNA polymerase and the other polymerases discussed in this book is its direct role in origin recognition. This is covered in some detail by Hay (this volume).

Genetics

Adenovirus DNA polymerase shares conserved sequence motifs I–V, VII, and the ExoI–III segments with other members of the α-polymerase

family. It does not appear to contain segments such as δ-region C found in cellular polymerases δ and ε and herpes and poxvirus enzymes (Zhang et al. 1991). Joung et al. (1991) tested the importance of conserved region I by site-directed mutagenesis and assays of enzyme activity from extracts of transfected cells. A variety of substitution mutations had similar effects—mainly highly deleterious—on initiation and elongation assays, with the conserved glycine being the most tolerant of alterations.

Extensive linker insertion mutagenesis of both subunits of adenovirus polymerase has been performed (Freimuth and Ginsberg 1986; Chen and Horwitz 1989; Fredman et al. 1991; Roovers et al. 1991, 1993). The major finding from these studies is that mutations at locations throughout either subunit destroy polymerase activity in both initiation and elongation assays. Although it is difficult to rule out effects of the mutations on overall protein folding, the results are in accord with the studies on herpesvirus and poxvirus enzymes which suggest that polymerase active sites require contributions from distant regions of the molecule. These mutations have also been used to investigate protein-protein interactions, as summarized by Hay (this volume).

A novel activity—attachment to nuclear matrix—has been ascribed to the terminal protein, and this is thought to be important as well for transcriptional efficiency of viral DNA, presumably by proper intranuclear localization (Schaack et al. 1990; Fredman and Engler 1993). pTP also appears to facilitate the nuclear localization of the catalytic subunit, in a study that also mapped both subunits' nuclear localization signals (Zhao and Padmanabhan 1988). A third novel activity, which remains unexplained, is the role of the polymerase in oncogenic transformation; a temperature-sensitive *pol* mutation confers a temperature-sensitive transformation phenotype on the virus toward rat cells (Miller and Williams 1987).

REGULATION OF VIRAL POLYMERASES

The expression of viral DNA polymerases is regulated during virus infection. Much of this regulation is transcriptional so that the genes are transcribed at the correct time; most polymerases are "early" gene products, expressed prior to and independent of DNA synthesis. A striking feature of many viral polymerases is that they are subject to posttranscriptional controls that down-regulate their abundance. The consequences of this kind of control are perhaps better understood in RNA virus and retrovirus (Meier et al. 1987; Felsenstein and Goff 1988; Dinman and Wickner 1992). There it appears that overexpression of polymerase can be deleterious due to inappropriate ratios of polymerase

to the proteins with which it interacts. Mechanisms in these and bacteriophage systems to set low polymerase abundance relative to other proteins include partial transcriptional termination, relatively inefficient translational frameshifting, nonsense suppression, and autogenous translational regulation (Iverson and Rose 1981; Andrake et al. 1988; Atkins et al. 1990; Tuerk et al. 1990). The viruses whose polymerases are described in this chapter also utilize posttranscriptional mechanisms to down-regulate polymerase abundance.

Translational Shutoff of HSV Pol

HSV pol is encoded by a single major mRNA on which the *pol* gene is the only known function (a small open reading frame [ORF] upstream of the *pol* ORF is not important for regulation) (Yager and Coen 1988; Yager et al. 1990). At times when the synthesis of most early proteins peaks, the mRNA is poorly translated, resulting in a shutoff of polymerase expression (Yager et al. 1990; Wobbe et al. 1993). The mRNA is poorly associated with polyribosomes at these times, suggesting a block at the level of translational initiation. Neither the *cis*-acting sequences nor the *trans*-acting factors for this shutoff are known. However, translational shutoff depends on viral DNA synthesis (Wobbe et al. 1993), suggesting that a protein synthesized late in infection is required. Oddly, when DNA synthesis is inhibited by drugs such as aphidicolin or by mutation, *pol* mRNA accumulation declines, leading to no net change in polymerase accumulation (Wobbe et al. 1993). Whether this effect on mRNA accumulation is transcriptional or posttranscriptional is unclear. Similar effects of aphidicolin on the accumulation of baculovirus *pol* mRNA have been noted (Tomalski et al. 1988).

Other Herpesvirus Pols

EBV DNA polymerase expression has a number of unusual features (Furnari et al. 1992). The *pol* gene lacks a TATA box and contains multiple transcriptional start sites. There is a functional poly(A) signal near the 5′ end of the gene; this could provide a mechanism for limiting polymerase expression. In the B95-8 strain, the 3′ end of *pol* mRNA is generated in the absence of a poly(A) signal. The mRNAs nevertheless are polyadenylated but contain a series of inserted nucleotides between the *pol* sequences and the poly(A) tail (Furnari et al. 1993). CMV *pol* mRNA contains a long 5′-untranslated region (Kouzarides et al. 1987), which impairs translation in transfected cells and in vitro (Geballe and Mocarski 1988; Ye and Huang 1993).

Vaccinia Virus Pol

The regulation of vaccinia virus polymerase expression is in some ways the mirror image of HSV regulation. McDonald et al. (1992) found that under normal circumstances, vaccinia *pol* mRNA is translated with equal efficiencies at all stages of infection. However, in the presence of aphidicolin, the mRNA, rather than declining late in infection, remains at high levels, but polymerase translation is shut off. The effect of aphidicolin does not seem to be on DNA synthesis, however, as aphidicolin-resistant *pol* mutants manifest the same effects. Whether polymerase expression is regulated differently from other early genes has not yet been fully explored.

Splicing Versus Polyadenylation Regulates Adenovirus Polymerase Synthesis

The mechanisms of posttranscriptional regulation of adenovirus DNA polymerase are perhaps the best understood. Pol, pTP, and DBP share the same promoter. The mRNAs for these proteins, however, differ greatly in abundance, such that the mRNA for DBP is roughly 100 times more abundant than those for pol and pTP (Stillman et al. 1981). The different mRNAs are generated by alternative splicing of a common primary transcript (Stillman et al. 1981; Shu et al. 1988). The *dbp* mRNA uses a poly(A) signal upstream of the ORFs for the two polymerase subunits, leading to the proposal that preferential use of this signal leads to low abundance of the *pol* and *pTP* mRNAs. Given our current understanding, this implies a race between splicing to generate the downstream mRNAs on the one hand, and cleavage, polyadenylation, and perhaps transcription termination on the other, with splicing losing the race most of the time. Thus, the ratio of the mRNAs and, by extension, the two polymerase subunits to DBP is set, perhaps exclusively, by a balance between two competing processes. Given the myriad protein-protein interactions of Pol, pTP, and DBP (Hay, this volume), it is presumably important to set the correct ratios between pol, pTP, and DBP to ensure proper stoichiometries. It is very tidy to utilize alternative splicing to set these ratios rather than setting transcription rates at three different promoters.

SUMMARY

Despite the many differences in replication strategies of the different virus groups and the numerous differences in enzymatic properties, there are major similarities that unite the different virus DNA polymerases.

The different polymerases all share sequence homologies with each other and with other members of the α-like DNA polymerase family. For each of the polymerases there is evidence against the idea that the polymerase activity resides on a single modular structural and functional domain. Each of the polymerases appears to require another viral protein to stimulate elongation, but by rather different mechanisms ranging from tightly associated subunits to utilization of proteins that are not subunits. In each case, there is evidence for posttranscriptional regulation of expression. Given the evolutionary relationships, it would be surprising if some of these unifying features among viral polymerases did not also apply to cellular enzymes.

ACKNOWLEDGMENTS

Thanks to the many colleagues, especially K. Biron, M. DePamphilis, P. Digard, J. Engler, A.K. Field, R. Hay, C. Knopf, I.R. Lehman, D. Marvel, G. Rohrmann, P. van der Vliet, K. Thompson, P. Traktman, T. Tsurumi, T. Wang, and D. Yager who generously provided advice, reprints and preprints, and personal communications. Thanks also to J. Brown, C. Chow, and M. Kramer for selfless help with figures, tables, and manuscript preparation. Grant support from the National Institutes of Health (AI-19838, AI-26077, and AI-33357) is gratefully acknowledged.

REFERENCES

Andrake, M., N. Guild, T. Hsu, L. Gold, C. Tuerk, and J. Karam. 1988. DNA polymerase of bacteriophage T4 is an autogenous translational repressor. *Proc. Natl. Acad. Sci.* **85:** 7942–7946.

Atkins, J.F., R.B. Weiss, and R.F. Gesteland. 1990. Ribosome gymnastics—Degree of difficulty 9.5, style, 10.0. *Cell* **62:** 413–423.

Bernad, A., Blanco, L., Lazaro, J.M., Martin, G., and Salas, M. 1989. A conserved 3′-5′ exonuclease active site in prokaryotic and eukaryotic DNA polymerases. *Cell* **59:** 219–228.

Berthomme, H., S.J. Monahan, D.S. Parris, B. Jacquemont, and A.L. Epstein. 1995. Cloning, sequencing, and functional characterization of the two subunits of the pseudorabies virus DNA polymerase holoenzyme: Evidence for specificity of interaction. *J. Virol.* **69:** 2811–2818.

Challberg, M.D. and P.T. Englund. 1979a. Purification and properties of the deoxyribonucleic acid polymerase induced by vaccinia virus. *J. Biol. Chem.* **254:** 7812–7819.

———. 1979b. The effect of template secondary structure on vaccinia DNA polymerase. *J. Biol. Chem.* **254:** 7820–7826.

Challberg, M.D. and T.J. Kelly. 1979. Adenovirus DNA replication in vitro. *Proc. Natl. Acad. Sci.* **76:** 655–659.

Chen, M. and M.S. Horwitz. 1989. Dissection of functional domains of adenovirus DNA polymerase by linker-insertion mutagenesis. *Proc. Natl. Acad. Sci.* **86:** 6116–6120.

Chiou, H.C., S.K. Weller, and D.M. Coen. 1985. Mutations in the herpes simplex virus major DNA-binding protein gene leading to altered sensitivity to DNA polymerase inhibitors. *Virology* **145:** 213–226.

Chiou, H.C., K. Kumura, A. Hu, K.M. Kerns, and D.M. Coen. 1995. Penciclovir-resistance mutations in the herpes simplex virus DNA polymerase gene. *Antiviral Chem. Chemother.* **6:** 281–288.

Chiou, J.-F. and Y.-C. Cheng. 1985. Interaction of Epstein-Barr virus DNA polymerase and 5′-triphosphates of several antiviral nucleoside analogs. *Antimicrob. Agents Chemother.* **27:** 416–418.

Chow, C.S. and D.M. Coen. 1995. Mutations that specifically impair the DNA binding activity of the herpes simplex virus protein UL42. *J. Virol.* **69:** 6965–6971.

Citarella, R.V., R. Muller, A. Schabach, and A. Weissbach. 1972. Studies on vaccinia virus-directed deoxyribonucleic acid polymerase. *J. Virol.* **10:** 721–729.

Crute, J.J. and I.R. Lehman. 1989. Herpes simplex-1 DNA polymerase: Identification of an intrinsic 5′–3′ exonuclease with ribonuclease H activity. *J. Biol. Chem.* **264:** 19266–19270.

Defillippes, F.M. 1989. Site of the base change in the vaccinia virus DNA polymerase gene which confers aphidicolin resistance. *J. Virol.* **63:** 4060–4063.

Derbyshire, V., M. Astatke, and C.M. Joyce. 1993. Re-engineering the polymerase domain of Klenow fragment and evaluation of overproduction and purification strategies. *Nucleic Acids Res.* **21:** 5439–5448.

Derse, D. and Y.-C. Cheng. 1981. Herpes simplex virus type 1 DNA polymerase: Kinetic properties of the associated 3′–5′ exonuclease activity and its role in araAMP incorporation. *J. Biol. Chem.* **256:** 8525–8530.

Derse, D., K.F. Bastow, and Y.-C. Cheng. 1982. Characterization of the DNA polymerases induced by a group of herpes simplex type I variants selected for growth in the presence of phosphonoformic acid. *J. Biol. Chem.* **297:** 10251–10260.

Digard, P., W.R. Bebrin, K. Weisshart, and D.M. Coen. 1993a. The extreme C-terminus of herpes simplex virus DNA polymerase is crucial for functional interaction with processivity factor UL42 and for viral replication. *J. Virol.* **67:** 398–406.

Digard, P., C.S. Chow, L. Pirrit, and D.M. Coen. 1993b. Functional analysis of the herpes simplex virus UL42 protein. *J. Virol.* **67:** 1159–1168.

Digard, P., K.P. Williams, P. Hensley, C.E. Dahl, and D.M. Coen. 1995. Specific inhibition of the herpes simplex virus DNA polymerase by helical peptides corresponding to the subunit interface. *Proc. Natl. Acad. Sci.* **92:** 1456–1460.

Dinman, J.D. and R.B. Wickner. 1992. Ribosomal frameshifting efficiency and gag/gag-pol ratio are critical for yeast M_1 double-stranded RNA virus propagation. *J. Virol.* **66:** 3669–3676.

Dorsky, D.I. and C.S. Crumpacker. 1988. Expression of herpes simplex virus type 1 DNA polymerase gene by in vitro translation and effects of gene deletions on activity. *J. Virol.* **62:** 3224–3232

———. 1990. Site-specific mutagenesis of a highly conserved region of the herpes simplex virus type 1 DNA polymerase gene. *J. Virol.* **64:** 1394–1397.

Dorsky, D.I. and C. Plourde. 1993. Resistance to antiviral inhibitors caused by the mutation S889A in the highly conserved 885-GDTDS motif of the herpes simplex virus type 1 DNA polymerase. *Virology* **195:** 831–835.

Earl, P.L., E.V. Jones, and B. Moss. 1986. Homology between DNA polymerase of poxviruses, herpesviruses, and adenoviruses: Nucleotide sequence of the vaccinia virus DNA polymerase gene. *Proc. Natl. Acad. Sci.* **83:** 3659–3663.

Enomoto, T., J.H. Lichy, J.E. Ikeda, and J. Hurwitz. 1981. Adenovirus replication in vitro: Purification of the terminal protein in a functional form. *Proc. Natl. Acad. Sci.* **78:** 6779–6783.

Ertl, P.F. and K.L. Powell. 1992. Physical and functional interaction of human cytomegalovirus DNA polymerase and its accessory protein (ICP36) expressed in insect cells. *J. Virol.* **66:** 4126–4133.

Felsenstein, K.M. and S.P. Goff. 1988. Expression of the gag-pol fusion protein of Moloney murine leukemia virus without gag protein does not induce virion formation or proteolytic processing. *J. Virol.* **62:** 2179–2182.

Field, J., R.M. Gronostajski, and J. Hurwitz. 1984. Properties of the adenovirus DNA polymerase. *J. Biol. Chem.* **259:** 9487–9495.

Frank, K.B. and Y.-C. Cheng. 1986. Inhibition of herpes simplex virus DNA polymerase by purine ribonucleoside monophosphates. *J. Biol. Chem.* **261:** 1510–1513.

Frank, K.B., D.D. Derse, K.F. Bastow, and Y.-C. Cheng. 1984. Novel interaction of aphidicolin with herpes simplex virus DNA polymerase and polymerase-associated exonuclease. *J. Biol. Chem.* **259:** 13282–13286.

Fredman, J.N. and J.A. Engler. 1993. Adenovirus precursor to terminal protein interacts with the nuclear matrix in vivo and in vitro. *J. Virol.* **67:** 3384–3395.

Fredman, J.N., S.C. Pettit, M.S. Horwitz, and J.A. Engler. 1991. Linker insertion mutations in the adenovirus preterminal protein that affect DNA replication in vivo and in vitro. *J. Virol.* **65:** 4591–4597.

Freimuth, P.I. and H.S. Ginsberg. 1986. Codon insertion mutant of the adenovirus terminal protein. *Proc. Natl. Acad. Sci.* **83:** 7816–7820.

Furnari, F.B., M.D. Adams, and J.S. Pagano. 1992. Regulation of the Epstein-Barr virus DNA polymerase gene. *J. Virol.* **66:** 2837–2845.

———. 1993. Unconventional processing of the 3′ termini of the Epstein-Barr virus DNA polymerase mRNA. *Proc. Natl. Acad. Sci.* **90:** 378–382.

Gao, M., S.F. DiTusa, and M.G. Cordingley. 1993. The C-terminal third of UL42, a HSV-1 DNA replication protein, is dispensable for viral growth. *Virology* **194:** 647–653.

Geballe, A.P. and E.S. Mocarski. 1988. Translational control of cytomegalovirus gene expression is mediated by upstream AUG codons. *J. Virol.* **62:** 3334–3340.

Gibbs, J.S., H.C. Chiou, K.F. Bastow, Y.C. Cheng, and D.M. Coen. 1988. Identification of amino acids in herpes simplex virus DNA polymerase involved in substrate and drug recognition. *Proc. Natl. Acad. Sci.* **85:** 6672–6676.

Gibbs, J.S., K. Weisshart, P. Digard, A. deBruynKops, D.M. Knipe, and D.M. Coen. 1991. Polymerization activity of an α-like DNA polymerase requires a conserved 3′–5′ exonuclease active site. *Mol. Cell. Biol.* **11:** 4786–4795.

Gottlieb, J. and M.D. Challberg. 1994. Interaction of herpes simplex virus type 1 DNA polymerase and the UL42 accessory protein with a model primer template. *J. Virol.* **68:** 4937–4945.

Gottlieb, J., A.I. Marcy, D.M. Coen, and M.D. Challberg. 1990. The HSV-1 UL42 gene product: A subunit of DNA polymerase that functions to increase processivity. *J. Virol.* **64:** 5976–5987.

Haffey, M.L., J. Novotny, R.E. Bruccoleri, R.D. Carroll, J.T. Stevens, and J.T. Matthews.

1990. Structure-function studies of the herpes simplex virus DNA polymerase. *J. Virol.* **64:** 5008–5018

Hall, J.D., P.A. Furman, M.H. St. Clair, and C.W. Knopf. 1985. Reduced in vivo mutagenesis by mutant herpes simplex DNA polymerase involves improved nucleotide selection. *Proc. Natl. Acad. Sci.* **82:** 3889–3993.

Hall, J.D., Y. Wang, J. Pierpont, M.S. Berlin, S.E. Rundlett, and S. Woodward. 1989. Aphidicolin resistance in herpes simplex virus type 1 reveals features of the DNA polymerase dNTP binding site. *Nucleic Acids Res.* **17:** 9231–9244.

Hall, J.D., D.M. Coen, B.L. Fisher, M. Weisslitz, S. Randall, R.E. Almy, P.T. Gelep, and P.A. Schaffer. 1984. Generation of genetic diversity in herpes simplex virus: An antimutator phenotype maps to the DNA polymerase locus. *Virology* **132:** 26–37.

Hamatake, R.K., M. Bifano, D.J. Tenney, W.W. Hurlburt, and M.G. Cordingley. 1993. The herpes simplex virus type 1 DNA polymerase accessory protein, UL42, contains a functional protease-resistant domain. *J. Gen. Virol.* **74:** 2181–2189.

Hart, G.J. and R.E. Boehme. 1992. The effect of the UL42 protein on the DNA polymerase activity of the catalytic subunit of the DNA polymerase encoded by herpes simplex virus type 1. *FEBS Lett.* **305:** 97–100.

Hernandez, T.R. and I.R. Lehman. 1990. Functional interaction between the herpes simplex-1 DNA polymerase and UL42 protein *J. Biol. Chem.* **265:** 11227–11232.

Hwang, C.B.C. and H.J.-H. Chen. 1995. An altered spectrum of herpes simplex virus mutations mediated by an antimutator DNA polymerase. *Gene* **152:** 191–193.

Hwang, C.B.C., K.L. Ruffner, and D.M. Coen. 1992. A point mutation within a distinct conserved region of the herpes simplex virus DNA polymerase gene confers drug resistance. *J. Virol.* **66**: 1774–1776.

Ito, J. and D.K. Brathwaite. 1991. Compilation and alignment of DNA polymerase sequences. *Nucleic Acids Res.* **19:** 4045–4057.

Iverson, L.E. and J.K. Rose. 1981. Localized attenuation and discontinuous synthesis during vesicular stomatitis virus transcription. *Cell* **23:** 477–484.

Joung, I., M.S. Horwitz, and J.A. Engler. 1991. Mutagenesis of conserved region I in the DNA polymerase from human adenovirus serotype 2. *Virology* **184:** 235–241.

Kiehl, A. and D.I. Dorsky 1991. Cooperation of EBV DNA polymerase and EA-D (BMRF1) in vitro and colocalization in nuclei of infected cells. *Virology* **184:** 330–340.

———. 1995. Bipartite DNA-binding region of the Epstein-Barr virus BMRF1 product essential for DNA polymerase accessory function. *J. Virol.* **69:** 1669–1677.

Knopf, C.W. and R. Strick. 1993. Herpes simplex virus type 1 DNA polymerase: Eukaryotic model enzyme and principal target of antiviral therapy. In *Frontiers of virology* (ed. G. Darai and Y. Becker), vol. 3, pp. 87–135. Springer Verlag, Heidelberg.

Knopf, C.W. and K. Weisshart. 1990. Comparison of exonucleolytic activities of herpes simplex virus type-1 DNA polymerase and DNase. *Eur. J. Biochem.* **191:** 263–273.

Kouzarides, T., A.T. Bankier, S.C. Satchwell, K. Weston, P. Tomlinson, and B.G. Barrell. 1987. Sequence and transcription analysis of the human cytomegalovirus DNA polymerase gene. *J. Virol.* **61:** 125–133.

Krokan, H., P. Schaffer, and M.L. DePamphilis. 1979. Involvement of eucaryotic deoxyribonucleic acid polymerase α in the replication of cellular and viral deoxyribonucleic acid. *Biochemistry* **18:** 4431–4443.

Larder, B.A., S.D. Kemp, and G. Darby. 1987. Related functional domains in virus DNA polymerases. *EMBO J.* **6:** 169–175.

Lichy, J.H., J. Field, M.S. Horwitz, and J. Hurwitz. 1981. Formation of a covalent com-

plex between the 80kDa adenovirus terminal protein and 5′-dCMP in vitro. *Proc. Natl. Acad. Sci.* **78:** 2678–2682.

———. 1982. Separation of the adenovirus terminal protein precursor from its associated DNA polymerase: Role of both proteins in the initiation of adenovirus DNA replication. *Proc. Natl. Acad. Sci.* **79:** 5225–5229.

Lin, J.-C., N.D. Sista, F. Besençon, J. Kamine, and J.S. Pagano. 1991. Identification and functional characterization of Epstein-Barr virus DNA polymerase by in vitro transcription-translation of a cloned gene. *J. Virol.* **65:** 2728–2731.

Lindenbaum, J.O., J. Field, and J. Hurwitz. 1986. The adenovirus DNA binding protein and adenovirus DNA polymerase interact to catalyze elongation of primed DNA templates. *J. Biol. Chem.* **261:** 10218–10227.

Loh, L.C., W.J. Britt, C. Raggo, and S. Laferté. 1994. Sequence analysis and expression of the murine cytomegalovirus phosphoprotein pp50, a homolog of the human cytomegalovirus UL44 gene product. *Virology* **200:** 413–427.

Longiaru, M., J.-E. Ikeda, Z. Jarkovsky, S.B. Horwitz, and M.S. Horwitz. 1979. The effect of aphidicolin on adenovirus DNA synthesis. *Nucleic Acids Res.* **6:** 3369–3386.

Lurain, N.S., L.K. Penland, and K.D. Thompson. 1994. Ganciclovir-resistant DNA polymerase mutants isolated from an HCMV clinical specimen. Abstr. 19th International Herpesvirus Workshop, #211.

Lurain, N.S., K.D. Thompson, E.W. Holmes, and G.S. Read. 1992. Point mutations in the DNA polymerase gene of human cytomegalovirus that result in resistance to antiviral agents. *J. Virol.* **66:** 7146–7152.

Mar, E.-C., J.-F. Chiou, Y.-C. Cheng, and E.-S. Huang. 1985. Inhibition of cellular DNA polymerase α and human cytomegalovirus-induced DNA polymerase by the triphosphates of 9-(2-hydroxyethoxymethyl)guanine and 9-(1,3,-dihdroxy-2-propoxymethyl)guanine. *J. Virol.* **53:** 776–780.

Marcy, A.I., C.B.C. Hwang, K.L. Ruffner, and D.M. Coen. 1990a. Engineered herpes simplex virus DNA polymerase point mutants: The most highly conserved region shared among α-like DNA polymerases is involved in substrate recognition. *J. Virol.* **64:** 5883–5890.

Marcy, A.I., P.D. Olivo, M.D. Challberg, and D.M. Coen. 1990b. Enzymatic activities of overexpressed herpes simplex virus DNA polymerase purified from recombinant baculovirus-infected insect cells. *Nucleic Acids Res.* **18:** 1207–1215.

Marsden, H.S., M. Murphy, G.L. McVey, K.A. MacEachran, A.M. Owsianka, and N.D. Stow. 1994. Role of the carboxy-terminus of HSV-1 DNA polymerase in its interaction with UL42. *J. Gen. Virol.* **75:** 3127–3135.

Martin, J.L., C.E. Brown, N. Matthews-Davis, and J.E. Reardon. 1994. Effects of antiviral nucleoside analogs on human DNA polymerases and mitochondrial DNA synthesis. *Antimicrob. Agents Chemother.* **38:** 2743–2749.

Matthews, J.T., B.J. Terry, and A.K. Field. 1993. The structure and function of the HSV replication proteins: Defining novel antiviral targets. *Antiviral Res.* **20:** 89–114.

McDonald, W.F. and P. Traktman. 1994a. Overexpression and purification of the vaccinia virus DNA polymerase. *Protein Expr. Purif.* **5:** 409–421.

———. 1994b. Vaccinia virus DNA polymerase: In vitro analysis of parameters affecting processivity. *J. Biol. Chem.* **269:** 31190–31197.

McDonald, W.F., V. Crozel-Goudot, and P. Traktman. 1992. Transient expression of the vaccinia virus DNA polymerase is an intrinsic feature of the early phase of infection and is unlinked to DNA replication and late gene expression. *J. Virol.* **66:** 534–547.

Meier, E., G.G. Harmison, and M. Schubert. 1987. Homotypic and heterotypic exclusion of vesicular stomatitis virus replication by high levels of recombinant polymerase protein L. *J. Virol.* **61:** 3133–3142.

Miller, B.W. and J. Williams. 1987. Cellular transformation by adenovirus type 5 is influenced by the viral DNA polymerase. *J. Virol.* **61:** 3630–3634.

Monahan, S.J., T.F. Barlam, C.S. Crumpacker, and D.S. Parris. 1993. Two regions of the herpes simplex virus type 1 UL42 protein are required for its functional interaction with the viral DNA polymerase. *J. Virol.* **67:** 5922–5931.

Morrison, A., J.B. Bell, T.A. Kunkel, and A. Sugino. 1991. Eukaryotic DNA polymerase amino acid sequence required for 3′–5′ exonuclease activity. *Proc. Natl. Acad. Sci.* **88:** 9473–9477.

Mul, Y.M. and P.C. van der Vliet. 1993. The adenovirus DNA binding protein effects the kinetics of DNA replication by a mechanism distinct from NF1 or Oct-1. *Nucleic Acids Res.* **21:** 641–647.

Nagata, K., R.A. Guggenheimer, and J. Hurwitz. 1983. Adenovirus DNA replication in vitro: Synthesis of full length DNA with purified proteins. *Proc. Natl. Acad. Sci.* **80:** 4266–4270.

Nagata, K., R.A. Guggenheimer, T. Enomoto, J.H. Lichy, and J. Hurwitz. 1982. Adenovirus DNA replication in vitro: Identification of a host factor that stimulates synthesis of the preterminal protein-dCMP complex. *Proc. Natl. Acad. Sci.* **79:** 6438–6442.

Nishiyama, Y., M. Koichiro, and S. Yoshida. 1983. Characterization of human cytomegalovirus-induced DNA polymerase and the associated 3′–5′ exonuclease. *Virology* **124:** 221–231.

Pedrali-Noy, G. and S. Spadari. 1980. Mechanism of inhibition of herpes simplex virus and vaccinia virus DNA polymerases by aphidicolin, a highly specific inhibitor of DNA replication in eukaryotes. *J. Virol.* **36:** 457–464.

Reardon, J.E. and T. Spector. 1989. Herpes simplex virus type 1 DNA polymerase: Mechanism of inhibition by acyclovir triphosphate. *J. Biol. Chem.* **264:** 7405–7411.

Reha-Krantz, L.J. 1990. Genetic evidence for two protein domains and a potential new activity in bacteriophage T4 DNA polymerase. *Genetics* **124:** 213–220.

Reid, R., E.-C. Mar, E.-S. Huang, and M.D. Topal. 1988. Insertion and extension of acyclic, dideoxy, and ara nucleotides by herpesviridae, human α, and human β polymerases. *J. Biol. Chem.* **263:** 3898–3904.

Roovers, D.J., F. van der Lee, J. van der Wees, and J.S. Sussenbach. 1993. Analysis of the adenovirus type 5 terminal protein precursor and DNA polymerase by linker insertion mutagenesis. *J. Virol.* **67:** 265–276.

Roovers, D.J., C.S.H. Young, H.L. Vos, and J.S. Sussenbach. 1991. Linker mutation scanning of the genes encoding the adenovirus type 5 terminal protein precursor and DNA polymerase. *Virology* **180:** 273–284.

Schaack, J., W.Y.-W. Ho, P. Freimuth, and T. Shenk. 1990. Adenovirus terminal protein mediates both nuclear matrix association and efficient transcription of adenovirus DNA. *Genes Dev.* **4:** 1197–1298.

Shu, L., S.C. Pettit, and J.A. Engler. 1988. The precise structure and coding capacity of mRNAs from early region 2B of human adenovirus serotype 2. *Virology* **165:** 348–356.

Simon, M., L. Giot, and G. Faye. 1991. The 3′–5′ exonuclease activity located in the DNA polymerase δ subunit of *Saccharomyces cerevisiae* is required for accurate replication. *EMBO J.* **8:** 2165–2170.

Skalka, A.-M. and S.P. Goff, eds. 1993. *Reverse transcriptase.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Stillman, B.W., J.B. Lewis, L.T. Chow, M.B. Mathews, and J.E. Smart. 1981. Identification of the gene and mRNA for the adenovirus terminal protein precursor. *Cell* **23:** 497–508.

Stow, N.D. 1993. Sequences at the C-terminus of the herpes simplex virus type 1 UL30 protein are dispensible for DNA polymerase activity but not for viral origin-dependent DNA replication. *Nucleic Acids Res.* **21:** 87–92.

Sullivan, V., K.K. Biron, C. Talarico, S.C. Stanat, M. Davis, L.M. Pozzi, and D.M. Coen. 1993. A point mutation in the human cytomegalovirus DNA polymerase gene confers resistance to ganciclovir and phosphonylmethoxyalkyl derivatives. *Antimicrob. Agents Chemother.* **37:** 19–25.

Taddie, J.A. and P. Traktman. 1991. Genetic characterization of the vaccinia virus DNA polymerase: Identification of point mutations conferring altered drug sensitivities and reduced fidelity. *J. Virol.* **65:** 869–879.

———. 1993. Genetic characterization of the vaccinia virus DNA polymerase: Cytosine arabinoside resistance requires a variable lesion conferring phosphonoacetate resistance in conjunction with an invariant mutation localized to the 3′–5′ exonuclease domain. *J. Virol.* **67:** 4323–4336.

Tenney, D.J., W.W. Hurlburth, M. Bifano, J.T. Stevens, P.A. Micheletti, R.K. Hamatake, and M.G. Cordingley. 1993a. Deletions of the carboxy terminus of herpes simplex virus type 1 UL42 define a conserved amino-terminus functional domain. *J. Virol.* **67:** 1959–1966.

Tenney, D.J., P.A. Micheletti, J.T. Stevens, R.K. Hamatake, J.T. Matthews, A.R. Sanchez, W.W. Hurlburt, M. Bifano, and M.G. Cordingley. 1993b. Mutations in the C terminus of herpes simplex virus type 1 DNA polymerase can affect binding and stimulation by its accessory protein UL42 without affecting basal polymerase activity. *J. Virol.* **67:** 543–547.

Tomalski, M.D., J. Wu, and L.K. Miller. 1988. The location, sequence, transcription, and regulation of a baculovirus DNA polymerase gene. *Virology* **167:** 591–600.

Traktman, P., M. Kelvin, and S. Pacheco. 1989. Molecular genetic analysis of vaccinia virus DNA polymerase mutants. *J. Virol.* **63:** 841–846.

Tsurumi, T. 1992. Selective inhibition of the 3′–5′ exonuclease activity associated with Epstein-Barr virus DNA polymerase by ribonucleoside 5′-monophosphates. *Virology* **189:** 803–807.

Tsurumi, T., T. Daikoku, and Y. Nishiyama. 1994. Further characterization of the interaction between the Epstein-Barr virus DNA polymerase catalytic subunit and its accessory subunit with regard to the 3′–5′ exonucleolytic activity and stability of initiation complex at primer-terminus. *J. Virol.* **68:** 3354–3363.

Tsurumi, T., T. Daikoku, R. Kurachi, and Y. Nishiyama. 1993a. Functional interaction between Epstein-Barr virus DNA polymerase catalytic subunit and its accessory subunit in vitro. *J. Virol.* **67:** 7648–7653.

Tsurumi, T., A. Kobayashi, K. Tamai, T. Daikoku, R. Kurachi, and Y. Nishiyama. 1993b. Functional expression and characterization of the Epstein-Barr virus DNA polymerase catalytic subunit. *J. Virol.* **67:** 4651–4658.

Tuerk, C., S. Eddy, D. Parma, and L. Gold. 1990. Autogenous translational operator recognized by bacteriophage T4 DNA polymerase. *J. Mol. Biol.* **213:** 749–761.

Wang, Y., S. Woodward, and J.D. Hall. 1992. Use of suppressor analysis to identify

DNA polymerase mutations in herpes simplex virus which affect deoxynucleoside triphosphate substrate specificity. *J. Virol.* **66:** 1814–1816.

Weiland, K.L, N.L. Oien, F. Homa, and M.W. Wathen. 1994. Functional analysis of human cytomegalovirus polymerase accessory protein. *Virus Res.* **34:** 191–206.

Weissbach, A., S.-C.L. Hong, J. Aucker, and R. Muller. 1973. Characterization of herpes simplex virus-induced deoxyribonucleic acid polymerase. *J. Biol. Chem.* **248:** 6270–6277.

Weisshart, K., A.A. Kuo, C.B.C. Hwang, K. Kumura, and D.M. Coen. 1994. Structural and functional organization of herpes simplex virus DNA polymerase investigated by limited proteolysis. *J. Biol. Chem.* **269:** 22788–22796.

Weisshart, K., A.A. Kuo, G.R. Painter, L.L. Wright, P.A. Furman, and D.M. Coen. 1993. Conformational changes induced in herpes simplex virus DNA polymerase upon DNA binding. *Proc. Natl. Acad. Sci.* **90:** 1028–1032.

Wobbe, K.K., P. Digard, D. Staknis, and D.M. Coen. 1993. Unusual regulation of expression of the herpes simplex virus DNA polymerase gene. *J. Virol.* **67:** 5419–5425.

Wong, S.W., A.F. Wahl, P.-M. Yuan, N. Arai, B.E. Pearson, K.-I. Arai, D. Korn, M.W. Hunkapiller, and T.S.-F. Wang. 1988. Human DNA polymerase α gene expression is cell proliferation dependent and its primary structure is similar to both prokaryotic and eukaryotic replicative DNA polymerases. *EMBO J.* **7:** 37–47.

Yager, D.R. and D.M. Coen. 1988. Analysis of the transcript of the herpes simplex virus DNA polymerase gene provides evidence that polymerase expression is inefficient at the level of translation. *J. Virol.* **62:** 2007–2015.

Yager, D.R., A.I. Marcy, and D.M. Coen. 1990. Translational regulation of herpes simplex virus DNA polymerase. *J. Virol.* **64:** 2217–2225.

Ye, L.-B. and E.-S. Huang. 1993. In vitro expression of the human cytomegalovirus DNA polymerase gene: Effects of sequence alterations on enzyme activity. *J. Virol.* **67:** 6339–6347.

Zhang, J., D.W. Chung, C.-K. Tan, K.M. Downey, E.W. Davie, and A.G. So. 1991. Primary structure of the catalytic subunit of calf thymus DNA polymerase δ: Sequence similarities with other DNA polymerases. *Biochemistry* **30:** 11742–11750.

Zhao, L.-J. and R. Padmanabhan. 1988. Nuclear transport of adenovirus DNA polymerase is facilitated by interaction with preterminal protein. *Cell* **55:** 1005–1015.