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Identification of amino acids in herpes simplex virus DNA polymerase involved in substrate and drug recognition

(sequencing of mutations/sequence similarities/antiviral chemotherapy/drug resistance/DNA polymerase α)

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ABSTRACT **Herpes simplex virus (HSV) encodes a DNA polymerase that is similar in several respects to the replicative mammalian DNA polymerase α. Recently, these and other DNA polymerases have been shown to share several regions of protein sequence similarity. Despite these similarities, antiviral drugs that mimic natural polymerase substrates specifically inhibit herpesvirus DNA polymerases. To study amino acids involved in substrate and drug recognition, we have characterized and mapped altered drug sensitivity markers of nine HSV *pol* mutants and sequenced the relevant portions of these mutants. The mutations were found to occur within four relatively small regions. One such region, which we designate region A, has sequence similarity only to DNA polymerases that are sensitive to certain antiviral drugs. The other three regions contain sequences that are similar among various DNA polymerases. The multiple mutations occurring within two of these regions make it likely that the regions interact directly with drugs and substrates. Our results lead us to favor a model in which protein folding allows interactions among the four regions to form the substrate and drug binding sites.**

DNA polymerases are central enzymes in DNA replication. In prokaryotic systems, studies of DNA polymerases have been greatly aided by model systems to which both genetic and biochemical methods can be applied. The herpes simplex virus (HSV) DNA polymerase, which is essential for HSV replication (1), provides such a model system for the study of eukaryotic DNA polymerases. This enzyme resembles the mammalian replicative DNA polymerase α (pol α) in several respects, including nuclear localization (2) and sensitivities to inhibitors such as aphidicolin (3–7) and dideoxythymidine triphosphate (4). Recently, HSV DNA polymerase (HSV pol), DNA polymerase I of yeast, a variety of animal and bacterial virus DNA polymerases, and pol α were shown to share six regions of striking sequence similarity (8–19). Of the viral polymerases sequenced to date, however, HSV pol appears most closely related to pol α (8). The six regions of sequence similarity have been designated regions I–VI by Wong *et al.* (8), with region I being most similar among the various polymerases and region VI being the least similar (Fig. 1). Such sequence similarities suggest conservation during evolution and important functional roles for these regions.

Despite these similarities, HSV pol is more sensitive than pol α to a number of inhibitors and thus serves as the ultimate target for a number of selective antiviral drugs. These include the pyrophosphate (PP_i) analogs phosphonoacetic acid (PAA) and phosphonoformic acid (20, 21), and the triphosphates of nucleoside analogs such as acyclovir (ACV), vidarabine, ganciclovir, and bromovinyldeoxyuridine (BVdU) (22–25). In addition, aphidicolin appears to inhibit HSV pol competitively with certain deoxynucleoside triphosphates (dNTPs) (5–7).

The availability of viral *pol* mutants that exhibit altered sensitivities to these drugs permits a unique combined pharmacological and genetic approach for investigating polymerase substrate recognition. Since the various drugs mimic and/or compete with the natural dNTP and PP_i substrates for enzyme binding (5–7, 22–26), mutations that alter polymerase sensitivity to these agents can be expected to identify amino acids involved in recognition of dNTPs and PP_i. We wished to determine whether any such amino acids were located in regions of sequence similarity with pol α and other viral DNA polymerases, indicating functional roles for these regions. On the other hand, identification and subsequent characterization of regions of HSV pol that differ from pol α, yet are involved in drug recognition, could aid in the design of more selective, targeted antiviral agents.

Since any given mutation might affect drug and substrate recognition indirectly, it is important to study enough mutations so that clusters might be revealed, suggesting direct participation of regions of amino acids in recognition. Toward this end, we have characterized the drug sensitivity phenotypes of a variety of HSV *pol* mutants derived from wild-type HSV type 1 strain KOS (23, 26–32), and we have mapped such altered drug sensitivity phenotypes from each mutant to a region of the *pol* gene of 1010 base pairs or less (ref. 9; H.C.C., J.S.G., and D.M.C., unpublished data). This has permitted us to use DNA sequencing to identify nine different amino acids involved in drug recognition, and presumably substrate recognition, and to compare their locations with the regions of sequence similarity between HSV pol and human pol α.

MATERIALS AND METHODS

Cloning and Sequencing of Mutants. Relevant properties of the mutants used and map locations of selected markers (H.C.C., J.S.G., and D.M.C., unpublished data) are shown in Table 1. For mutants tsD9 and PFA^r2, viral DNA, prepared as described (9), was digested with *Eco*RI, and the *Eco*RI M fragment (map units 0.422–0.448) was isolated as described (9). The *Eco*RI M fragment was then further cut with *Xho* I and *Kpn* I and ligated into M13mp18 and M13mp19 digested with *Sal* I and *Kpn* I. The *Eco*RI M fragment of mutant PAA^r5 was digested with *Kpn* I and *Bam*HI and ligated into similarly digested M13mp18 and M13mp19. For each of the other mutants, the isolated *Eco*RI M fragment was ligated into *Eco*RI-digested bacteriophage M13mp19 DNA. Phage containing inserts in each orientation were derived for each mutant, and relevant portions of the resulting single-stranded

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Abbreviations: HSV, herpes simplex virus; HSV pol, HSV DNA polymerase; pol α, DNA polymerase α; ACV, acyclovir; PAA, phosphonoacetic acid; BVdU, bromovinyldeoxyuridine.

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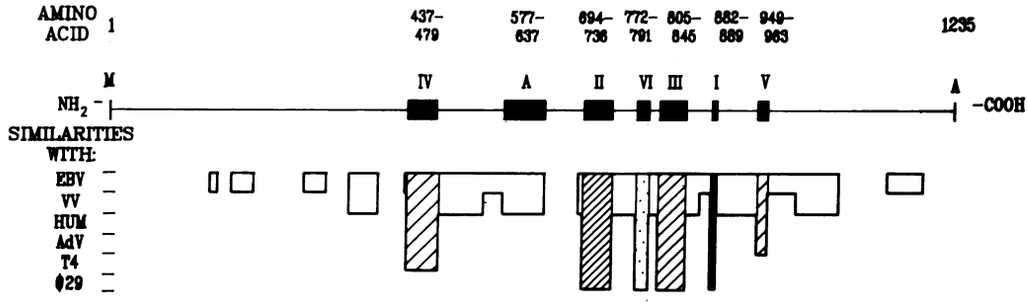

FIG. 1. Regions of sequence similarity of HSV pol with other DNA polymerases. Top line, HSV *pol* predicted protein product. Solid boxes, regions I–VI of sequence similarity described by Wong *et al.* (8), and region A described in this paper. Boxed areas below indicate protein sequence similarities shared among HSV and the indicated DNA polymerases. Areas corresponding to regions I–VI are indicated by degrees of shading roughly corresponding to the degree of sequence similarity found among the various polymerases, with region I having the most and region VI having the least amount of shading. The DNA polymerases listed are arranged according to their degree of sequence similarity with HSV. EBV, Epstein–Barr virus; VV, vaccinia virus; Hum, human DNA polymerase α; AdV, adenovirus; T4, bacteriophage T4; ϕ29, bacteriophage ϕ29.

DNAs were sequenced with an M13 universal primer, and, when necessary, oligonucleotide primers were designed by using the wild-type HSV strain KOS sequence. The following synthetic oligonucleotides were used: GGAGACCGCCGGCCGGCACGT (amino acid positions 688–694), GGTTCACGTGAAACCCGG (positions 712–706), TCAAGGTCGTGTGTAAC (positions 810–815), GTTACACACGACCTTGA (positions 815–810), CGCGCTGTTTCTGCCCC (positions 915–921), and TCAGCAGCAGCTTGGTG (positions 936–930). Sequencing was performed by the chain termination method (34) essentially as described (9), with Sequenase (United States Biochemical, Cleveland) (35) used in some reactions according to the manufacturer's recommendations.

RESULTS

Overview of Mutation Locations and Properties. Table 1 summarizes phenotypic and mapping data for nine HSV *pol* mutants. Because these mutants were derived by five different regimens and because they exhibit distinct phenotypic properties, we expected to identify different mutations in each mutant. Altered drug sensitivity markers in each mutant were mapped to specific regions of the *pol* gene by marker rescue techniques. These studies allowed localization of the mutational changes responsible for altered drug sensitivities to regions encoding 229 amino acids or less and will be reported separately (H.C.C., J.S.G., and D.M.C., unpublished data). Altered drug sensitivity markers that have been demonstrated by marker rescue to map exclusively to particular regions of the *pol* gene are listed in Table 1. We report here the sequence changes responsible for altered drug sensitivities. Rather than sequence the entire *pol* gene from each mutant, only the portion of each mutant *pol* gene to which altered drug sensitivity markers mapped was sequenced, as listed in Table 1. Each mutant had a unique amino acid change that is responsible for particular alterations in drug sensitivity. As shown in Fig. 2, these mutations are located in four relatively small regions within the HSV *pol* gene, three of which correspond to regions of sequence similarity shared with cellular and viral DNA polymerases.

By analysis of recombinant viruses derived by marker rescue, three of the mutants, ACG^{r}4, tsD9, and AraA^{r}9, must carry additional mutations that map outside the regions sequenced but contribute to the phenotypes associated with these mutants (H.C.C., J.S.G., and D.M.C., unpublished data). The phenotypes that we have assigned to individual mutations in these mutants may be modified by the presence of the additional still unmapped mutations. It should be possible to use recombinants derived in mapping experiments to locate the additional mutations.

Mutations in a Region Containing Little or No Sequence Similarity with pol α. The first group of mutations, shown in Fig. 2*A*, includes those in tsD9 and PFA^{r}2. These mutations

Table 1. Phenotypes of and mutations in the mutants studied

Mutant	Markers mapped				Map location	Region sequenced	Nucleotide change	Amino acid change
	ACV	PAA	Aph	Other				
tsD9	r	r/s		ts	536–696	536–696	G-2122 to A	Glu-597 to Lys
PFA^{r}2	r	r	hs		588–696*	536–925	A-2029 to G, C-2147 to T	Thr-566 to Ala, Ala-605 to Val
PFA^{r}1	r	r	hs/s		696–925	696–925	A-2431 to G†	Arg-700 to Gly
PFA^{r}5	r	r	hs		696–925	696–925	G-2504 to A	Ser-724 to Asn
PAA^{r}C	r	r	hs		696–925	696–925	C-2722 to A	Pro-797 to Thr
AraA^{r}13	r	r	hs	BVdU	696–925	696–925	G-2770 to A	Val-813 to Met
ACG^{r}4			hs/s		696–925	696–925	C-2795 to T	Thr-821 to Met
PAA^{r}5	r	r	hs		696–925	696–925	C-2857 to A	Arg-842 to Ser
AraA^{r}9	r	r/s	hs/s	DHPG^{hs}AraA^r	925–1008	925–1008	C-3216 to A	Asn-961 to Lys

Coordinates of marker locations and regions sequenced are listed in terms of the amino acid sequence of the *pol* major open reading frame. Map locations were determined in ref. 9 and by H.C.C., J.S.G., and D.M.C. (unpublished data). Only a subset of the altered drug sensitivity phenotypes are listed. The nucleotide changes listed are based on the distance from the center of the palindrome at the origin of replication, ori_L. The major *pol* transcript initiates at approximately nucleotide 126 (33). ACV, acyclovir; Aph, aphidicolin; ts, temperature-sensitive; r, resistant; s, sensitive; hs, hypersensitive; DHPG, ganciclovir; araA, vidarabine.

*The original map coordinates for the Aph^{hs} marker of PFA^{r}2 (9) were incorrect.

†An error in ref. 9 at nucleotide 2432 (A instead of the correct G) resulted in a lysine being mistakenly reported at amino acid 700 instead of the correct arginine. Similarly, nucleotide 2424 was reported to be A instead of the correct G; however, this does not result in an amino acid change.

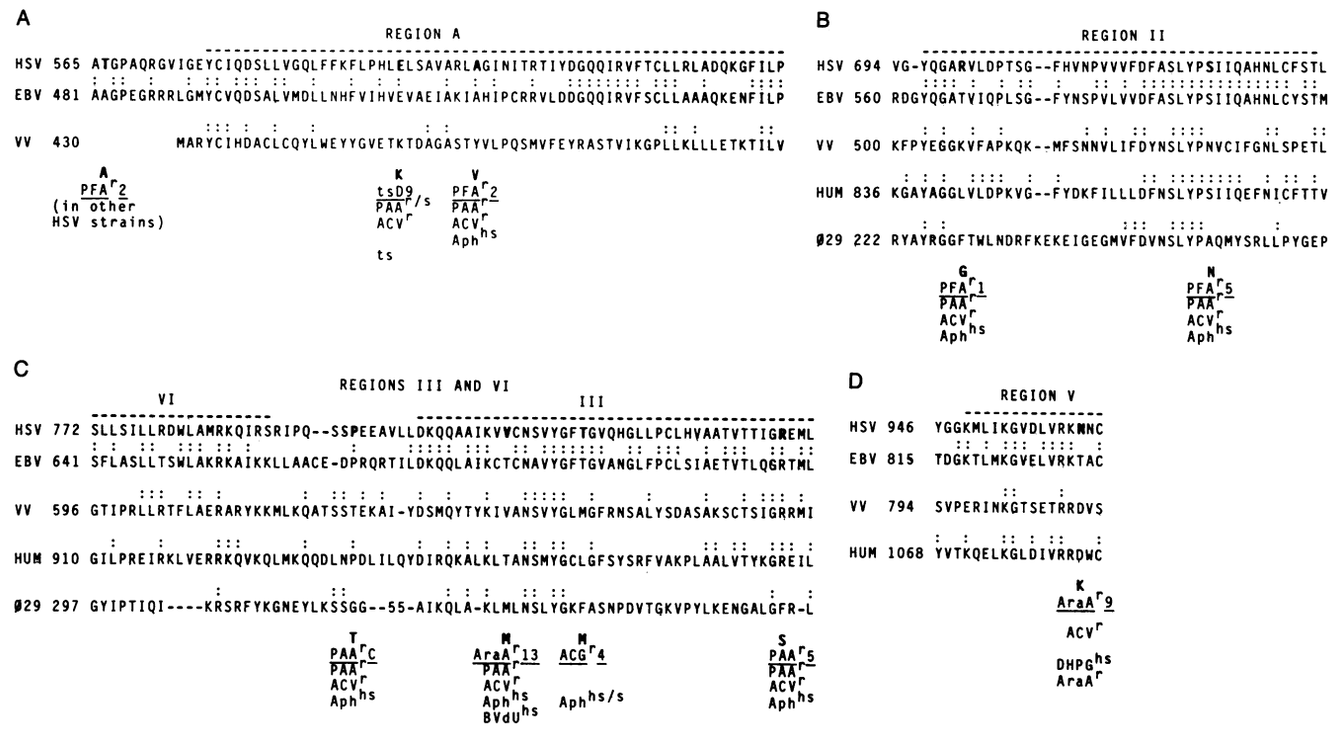

FIG. 2. Locations of HSV *pol* mutations that affect drug sensitivity. Alignment of select polymerases sharing sequence similarity is presented. Amino acids identical to that found in HSV are indicated with a colon. Gaps of amino acids that aid in alignment are indicated by hyphens. Shown are the groups of mutations that lie in or near region A (*A*), region II (*B*), regions III and VI (*C*), and region V (*D*). The limits of the regions are indicated by the dashed line. Relevant phenotypes conferred by the designated mutations are listed under the names of the mutants. Amino acids are designated by the single-letter code. Abbreviations are the same as in Table 1.

alter amino acids within a region of the HSV *pol* gene that shares little or no sequence similarity with human pol α (8). We designate this region A. Region A does contain sequences similar to those found in *pol* genes of other herpesviruses (e.g., Epstein–Barr virus; Fig. 2*A*) and, to a lesser extent (20% identity; Fig. 2*A*), vaccinia virus (10–12, 14, 17, 18). The limits of region A are defined by the sites at which the HSV and vaccinia virus sequences diverge. The mutation in tsD9, which confers resistance to ACV, marginal resistance to PAA, and temperature sensitivity, entails a nonconservative change from an acidic amino acid (glutamic acid) to a basic amino acid (lysine) at position 597. Interestingly, a lysine is found in the corresponding position in the vaccinia virus DNA polymerase. Mutant PFA^{r}2 contains two mutations in this vicinity, a threonine to alanine substitution at position 566 and a more conservative alanine to valine substitution at position 605. We predicted that the latter mutation would prove more important to the drug-resistance phenotype because an alanine is found at position 566 in several wild-type strains of HSV-1 (36–38). This prediction was verified by marker rescue analysis with DNA fragments generated by a restriction enzyme that cuts the *pol* gene between these two positions (H.C.C., J.S.G., and D.M.C., unpublished data). Thus, the conservative alanine to valine change at position 605 accounts for this mutant's resistance to PAA, ACV, and hypersensitivity to aphidicolin. The amino acids altered in tsD9 and PFA^{r}2 in region A occur at residues that are conserved in all the other herpesvirus DNA polymerases that have been sequenced to date (10–12, 18).

Mutations in Region II. Two of the mutations lie within region II, one of six regions that share sequence similarity with human pol α, yeast DNA polymerase I, and various viral DNA polymerases (8) (Fig. 2*B*). The mutation in PFA^{r}1 that confers resistance to PAA, ACV, and marginal hypersensitivity to aphidicolin is a relatively nonconservative arginine to glycine change at position 700. This position is highly variable among the cellular and viral polymerases. In contrast, the mutation in mutant PFA^{r}5 that confers resistance to PAA, ACV, and hypersensitivity to aphidicolin is a conservative serine to asparagine change at position 724. The serine at this position is found in all the herpesvirus DNA polymerases and almost every other polymerase that shares this region of sequence similarity (8–19, 39). Asparagine is found at this position in the vaccinia virus DNA polymerase.

Mutations in Sequences Including Region III. Four of the mutations lie in a segment that includes the region of sequence similarity that has been designated region III by Wong *et al.* (8) (Fig. 2*C*). Three of these lie within region III itself. The mutation in AraA^{r}13 that confers resistance to PAA, ACV, and the ACV congener buciclovir (28), and hypersensitivity to aphidicolin and the nucleoside analog BVdU, is a conservative valine to methionine change at position 813. Methionine is found at this position in the ϕ29 DNA polymerase (15). This position is highly variable among the cellular and viral polymerases; however, it is two amino acids away from an invariant lysine at position 811 and an invariant asparagine at position 815. The mutation in ACG^{r}4 that confers marginal hypersensitivity to aphidicolin is a relatively nonconservative threonine to methionine change at position 821. A threonine at this position is found in all other herpesvirus DNA polymerases (10–12, 18, 39). Methionine is found at this position in vaccinia virus DNA polymerase. The mutation in PAA^{r}5 that confers resistance to PAA and ACV and hypersensitivity to aphidicolin is a relatively nonconservative arginine to serine change at position 842. Arginine has been found at this position in all of the herpesvirus DNA polymerases (10–12, 18, 39) and in several of the other viral and cellular polymerases examined thus far.

The fourth mutation in this region, which lies just outside the stated boundaries of region III, is found in mutant PAA^{r}C and is a relatively nonconservative proline to threonine change at position 797. This mutation confers resistance to

PAA and ACV and hypersensitivity to aphidicolin. Position 797 in HSV pol lies only seven amino acids upstream from the stated N-terminal boundary of region III and seven amino acids downstream from the stated C-terminal boundary of the sequence designated as region VI (8). A proline is found in a similar position in human pol α, yeast DNA polymerase I, and in several herpesvirus DNA polymerases (8, 10, 12, 18, 39). A threonine is found in a similar position in vaccinia virus DNA polymerase (14).

A Mutation in Region V. The mutation in AraA^{r}9 that confers hypersensitivity to ganciclovir, and resistance to ACV and vidarabine lies within the sequence designated as region V by Wong *et al.* (8) (Fig. 2*D*). This mutation is a relatively nonconservative asparagine to lysine change at position 961. The amino acids at this position vary among asparagine, aspartic acid, and threonine in the cellular and viral polymerases that share this sequence; however, the amino acids two positions upstream and downstream are the same in most of the viral and cellular polymerases that share region V.

DISCUSSION

A Variety of Amino Acid Substitutions Can Confer Altered Drug Sensitivity. We have identified nine different amino acid substitutions that confer altered drug sensitivity. We found no preference for conservative mutations or mutations at amino acids that are shared among most DNA polymerases or among herpesvirus DNA polymerases. In contrast, Larder *et al.* (37) found that five of six *pol* lesions in ACV-resistant mutants were conservative substitutions in amino acids shared by other herpesvirus DNA polymerases.

Generally, HSV mutants that are resistant to PP_i analogs are hypersensitive to aphidicolin (29, 32). Of six such mutations in this study, two (PAA^{r}5 and PFA^{r}1) correspond to the suggestion (3) that this could be due to substitutions of basic amino acids to more neutral amino acids, which might lessen the affinity for acidic compounds such as PAA and increase the affinity for hydrophobic aphidicolin. Perhaps the other four changes alter the protein such that nearby basic amino acids are less accessible to the drugs. Alternatively, recognition of PP_i analogs and aphidicolin may depend on more than the basic or neutral nature of amino acid side chains.

Substitutions to Amino Acids Found in Other DNA Polymerases. Four of the mutations that confer aphidicolin hypersensitivity change amino acids to those found at the analogous position in vaccinia virus DNA polymerase or ϕ29 DNA polymerase. Despite this, both vaccinia virus and ϕ29 DNA polymerases are substantially less sensitive to aphidicolin than is wild-type HSV DNA polymerase (5, 40). This indicates that other amino acid sequence differences among these viral polymerases account for their relative aphidicolin sensitivities; such other differences may be viewed as naturally occurring second-site suppressor mutations.

Grouping of Mutations Conferring Altered Drug Sensitivity. The rather large number of amino acid substitutions that can cause alterations in drug sensitivity fall within four relatively small regions in the HSV *pol* gene. The grouping is strengthened by the inclusion of five other *pol* mutations from drug-resistant mutants identified by other groups (37, 38, 41). Since the drugs to which the mutants exhibit altered sensitivity mimic and/or compete with natural dNTP and PP_i substrates, these regions—A, II, III, and V—appear to be involved, directly or indirectly, in binding both the drugs and the corresponding substrates. The altered drug sensitivity mutations fall between amino acids 597 and 961, appearing to confirm our original proposal (9) that this portion of the HSV pol polypeptide folds to form part of a domain containing substrate and drug binding sites.

There has been one report of a drug-resistant *pol* mutant containing a mutation outside this portion of the *pol* gene. This mutant, TP2.5, contains the same serine to asparagine mutation at position 724 that we have identified in mutant PFA^{r}5 along with a glycine to aspartic acid change at position 335 (37) and exhibits altered drug sensitivity phenotypes similar to those of PFA^{r}5 (42). Thus, the mutation at position 724 seems sufficient to account for the phenotypes of TP2.5 (this report; H.C.C., J.S.G., and D.M.C., unpublished data).

Regions II and III Are Likely to Participate Directly in Substrate Recognition. Of the nine altered drug sensitivity mutations identified in this study, two lie within region II and three lie within region III (Fig. 2 *B* and *C*). Four other HSV *pol* mutations in drug-resistant mutants also lie within regions II and III (37, 38, 41). These are a tyrosine to histidine change in a HSV type 2 mutant at position 696 that confers aphidicolin resistance and hypersensitivity to PAA (41), an alanine to valine change at position 719 in two mutants that are PAA and ACV resistant (37, 38), an asparagine to serine mutation at position 815 in three ACV-resistant mutants that differ with respect to sensitivities to other drugs, and a glycine to serine change at position 841 in a fourth ACV-resistant mutant (37). Thus, there is a cluster of four mutations in region II and a cluster of five mutations in region III. Given this grouping and the high degree of sequence similarity among these regions in evolutionarily distant cellular and viral DNA polymerases, we consider it likely that amino acids within these regions directly interact with substrates and drugs.

The mutation in PAA^{r}C lies between sequences designated as region III and region VI (Fig. 2*C*), at a position only 7 amino acids from each region. In HSV pol, these two regions are separated by only 13 amino acids, leaving open the possibility that these two regions define a single continuous sequence. In other DNA polymerases, however, these regions are separated by longer variable stretches of primary structure, ranging up to >60 amino acids (8). This gives rise to the suggestion that the mutation in PAA^{r}C might confer altered drug sensitivity by altering HSV pol tertiary structure such that region III is shifted relative to other portions of the polymerase structure. That this mutation changes a proline to an amino acid less likely to bend the polypeptide chain lends strength to this suggestion.

Region V. Thus far, only one altered drug sensitivity mutation, that of AraA^{r}9, lies within the region of sequence similarity designated region V. This result indicates a functional role for region V. However, because any single mutation could exert its effects on substrate and drug recognition indirectly, we cannot yet say that it is likely that region V interacts directly with substrates or drugs.

Roles of Other Regions of Sequence Similarity. No mutations altering drug sensitivity have been found in regions I, IV, and VI. It is especially interesting that this is true for the region of greatest sequence similarity, region I. Since there are a number of amino acids in these regions that are highly variable among the viral and cellular polymerases, it seems possible that these amino acids could be changed without lethal effects. The construction of site-directed mutations in these regions may be required to elucidate their roles.

Region A: A Region Involved in Drug Recognition That Is Specific to Viruses That Are Sensitive to Certain Antiviral Drugs. Two altered drug sensitivity mutations, tsD9 and PFA^{r}2, identify region A, a region of the HSV pol that is involved in recognition of antiviral drugs but is not found in human pol α. A third mutation, which has been reported to confer resistance to PAA and ACV and hypersensitivity to aphidicolin (43), also lies within this region, substituting aspartic acid for glutamic acid at position 597 (37), the same position that is altered in tsD9. Sequences highly similar to this region are found in other herpesvirus DNA polymerases, which generally are more sensitive to the inhibitors PAA,

phosphonoformic acid, and the triphosphate derivatives of ACV, ganciclovir, vidarabine, and BVdU than is human pol α (44–47). A somewhat similar sequence can also be found in vaccinia virus DNA polymerase, which appears to be relatively sensitive to PAA, phosphonoformic acid, and ACV triphosphate (48–50); however, adenovirus DNA polymerase does not appear to contain such a sequence and adenovirus DNA replication seems to be relatively resistant to PAA (49). Thus, this region appears to be specific to viruses that are sensitive to certain antiviral drugs. We suggest that this region interacts either with drugs and substrates or with other regions of the polymerase in a way that allows viral DNA polymerases to be more sensitive to various antiviral drugs than human pol α. Further understanding of such interactions could lead to design of more targeted antiviral drugs.

Individual Regions vs. Interacting Regions in Forming Binding Sites. When these studies began, we thought that it might be possible to identify stretches of amino acids involved solely in dNTP binding, because certain mutants (30, 31, 42) exhibit altered sensitivity to nucleoside analogs, but little or none to PP_i analogs. Indeed, Larder *et al.* (37) have hypothesized that region III sequences have a direct role in nucleotide binding rather than in pyrophosphate exchange based on a mutation in that region found in three mutants that are highly resistant to ACV and 2- to 5-fold more sensitive to PAA than their wild-type parent. However, mutants in region III, including mutant AraA^{r}13 in the core of the sequence similarity, can be as resistant to PAA as mutants in region II, which Larder *et al.* (37) hypothesized tentatively to be the PP_i binding site.

In the three regions we have identified that contain more than one mutation, given mutations can confer altered sensitivity to PP_i analogs, nucleoside analogs, and/or aphidicolin. We cannot therefore identify any region as the sole binding site for any of the drugs or substrates. Instead, we favor a model in which interactions among the various regions help form the binding sites for PP_i and dNTPs. Such a model predicts that one should be able to isolate mutations in one region that can suppress temperature-sensitive or altered drug sensitivity mutations in another region. We have isolated partial revertants of certain of the mutants studied here, and their analysis, as well as physical studies, should be useful in determining specific amino acid residues that interact at the substrate binding sites in the protein.

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1. Aron, G. M., Purifoy, D. J. M. & Schaffer, P. A. (1975) *J. Virol.* **16**, 498–507.
2. Weissbach, A., Hong, S.-C. L., Aucker, J. & Muller, R. (1973) *J. Biol. Chem.* **248**, 6270–6277.
3. Frank, K. B. & Cheng, Y.-C. (1985) *Antimicrob. Agents Chemother.* **27**, 445–448.
4. Krokan, H., Schaffer, P. & DePamphilis, M. L. (1979) *Biochemistry* **18**, 4431–4443.
5. Pedrali-Noy, G. & Spadari, S. (1980) *J. Virol.* **36**, 457–464.
6. DiCioccio, R. A., Chadha, K. & Srivastava, B. I. S. (1980) *Biochim. Biophys. Acta* **609**, 224–231.
7. Frank, K. B., Derse, D. D., Bastow, K. F. & Cheng, Y.-C. (1984) *J. Biol. Chem.* **259**, 13282–13286.
8. Wong, S. W., Wahl, A. F., Yuan, P.-M., Arai, N., Pearson, B. E., Arai, K.-I., Korn, D., Hunkapiller, M. W. & Wang, T. S.-F. (1988) *EMBO J.* **7**, 37–47.
9. Gibbs, J. S., Chiou, H. C., Hall, J. D., Mount, D. W., Retondo, M. J. & Coen, D. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7969–7973.
10. Baer, R., Bankier, A. T., Biggin, M. D., Deininger, P. L., Farrell, P. J., Gibson, T. J., Hatfull, G., Hudson, G. S., Satchwell, S. C., Seguin, C., Tuffnell, P. S. & Barrell, B. G. (1984) *Nature (London)* **310**, 207–211.
11. Davison, A. J. & Scott, J. E. (1986) *J. Gen. Virol.* **67**, 1759–1816.
12. Kouzarides, T., Bankier, A. T., Satchwell, S. C., Weston, K., Tomlinson, P. & Barrell, B. G. (1987) *J. Virol.* **61**, 125–133.
13. Gingeras, T. R., Sciaky, D., Gelinas, R. E., Bing-Dong, J., Yen, C. E., Kelly, M. M., Bullock, P. A., Parsons, B. L., O'Neill, K. E. & Roberts, R. J. (1982) *J. Biol. Chem.* **257**, 13475–13491.
14. Earl, P. L., Jones, E. V. & Moss, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3659–3663.
15. Yoshikawa, H. & Ito, J. (1982) *Gene* **17**, 323–335.
16. Jung, G., Leavitt, M. C., Hsieh, J.-C. & Ito, J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8287–8291.
17. Hall, J. D. (1988) *Trends Genet.* **4**, 42–46.
18. Tsurumi, T., Maeno, K. & Nishiyama, Y. (1987) *Gene* **52**, 129–137.
19. Argos, P., Tucker, A. D. & Philipson, L. (1986) *Virology* **149**, 208–216.
20. Mao, J. C.-H., Robishaw, E. E. & Overby, L. R. (1975) *J. Virol.* **15**, 1281–1283.
21. Reno, J. M., Lee, L. F. & Boezi, J. A. (1978) *Antimicrob. Agents Chemother.* **13**, 188–192.
22. Furman, P. A., St. Clair, M. H., Fyfe, J. A., Rideout, J. L., Keller, P. M. & Elion, G. B. (1979) *J. Virol.* **32**, 72–77.
23. Coen, D. M., Furman, P. A., Gelep, P. T. & Schaffer, P. A. (1982) *J. Virol.* **41**, 909–918.
24. Germershausen, J., Bostedor, R., Field, A. K., Perry, H., Liou, R., Bull, H., Tolman, R. L. & Karkas, J. D. (1983) *Biochem. Biophys. Res. Commun.* **116**, 360–367.
25. St. Clair, M. H., Miller, W. H., Miller, R. L., Lambe, C. U. & Furman, P. A. (1984) *Antimicrob. Agents Chemother.* **25**, 191–194.
26. Derse, D., Bastow, K. F. & Cheng, Y.-C. (1982) *J. Biol. Chem.* **257**, 10251–10260.
27. Coen, D. M., Aschman, D. P., Gelep, P. T., Retondo, M. J., Weller, S. K. & Schaffer, P. A. (1984) *J. Virol.* **49**, 236–247.
28. Chiou, H. C., Kerns, K. M. & Coen, D. M. (1986) *Antimicrob. Agents Chemother.* **30**, 502–504.
29. Coen, D. M., Furman, P. A., Aschman, D. P. & Schaffer, P. A. (1983) *Nucleic Acids Res.* **11**, 5287–5297.
30. Furman, P. A., Coen, D. M., St. Clair, M. H. & Schaffer, P. A. (1981) *J. Virol.* **40**, 936–941.
31. Coen, D. M., Fleming, H. E., Leslie, L. K. & Retondo, M. J. (1985) *J. Virol.* **53**, 477–488.
32. Bastow, K. F., Derse, D. D. & Cheng, Y.-C. (1983) *Antimicrob. Agents Chemother.* **23**, 914–917.
33. Yager, D. R. & Coen, D. M. (1988) *J. Virol.* **62**, 2007–2015.
34. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
35. Tabor, S. & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4767–4771.
36. Quinn, J. P. & McGeoch, D. J. (1985) *Nucleic Acids Res.* **13**, 8143–8163.
37. Larder, B. A., Kemp, S. D. & Darby, G. (1987) *EMBO J.* **6**, 169–175.
38. Knopf, C. W. (1986) *Nucleic Acids Res.* **14**, 8225–8226.
39. Owen, L. J. & Field, H. J. (1988) *Arch. Virol.* **98**, 27–38.
40. Blanco, L. & Salas, M. (1986) *Virology* **153**, 179–187.
41. Tsurumi, T., Maeno, K. & Nishiyama, Y. (1987) *J. Virol.* **61**, 388–394.
42. Larder, B. A. & Darby, G. (1986) *Antimicrob. Agents Chemother.* **29**, 894–898.
43. Larder, B. A., Lisle, J. J. & Darby, G. (1986) *J. Gen. Virol.* **67**, 2501–2506.
44. Ruth, J. L. & Cheng, Y.-C. (1981) *Mol. Pharmacol.* **20**, 415–422.
45. Kallin, B., Sternas, L., Saemundssen, A. K., Luka, J., Jornvall, H., Eriksson, B., Tao, P.-Z., Nilsson, M. T. & Klein, G. (1985) *J. Virol.* **54**, 561–568.
46. Freitas, V. R., Smee, D. F., Chernow, M., Boehme, R. & Matthews, T. R. (1985) *Antimicrob. Agents Chemother.* **28**, 240–245.
47. Wahren, B., Ruden, U., Gadler, H., Oberg, B. & Eriksson, B. (1985) *J. Virol.* **56**, 996–1001.
48. Darby, G., Larder, B. A., Bastow, K. F. & Field, A. K. (1980) *J. Gen. Virol.* **48**, 451–454.
49. Bolden, A., Aucker, J. & Weissbach, A. (1975) *J. Virol.* **16**, 1584–1592.
50. Overby, L. R., Duff, R. G. & Mao, J. C.-H. (1977) *Ann. N.Y. Acad. Sci.* **284**, 310–320.