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Identification of New Protein Kinase-Related Genes in Three Herpesviruses, Herpes Simplex Virus, Varicella-Zoster Virus, and Epstein-Barr Virus

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By using amino acid sequence patterns (motifs) diagnostic of conserved regions within the catalytic domains of protein kinases, homologous open reading frames of three herpesviruses were identified as protein kinase-related genes. The three sequences, herpes simplex virus gene UL13, varicella-zoster virus gene 47, and Epstein-Barr virus gene BGLF4, resemble serine/threonine kinases rather than tyrosine kinases.

The phosphorylation of cellular and viral proteins observed during lytic infection of cells by herpesviruses appears to involve a number of different protein kinase (PK) activities (3, 10, 19–21, 25, 26, 29, 31, 36). Since it is difficult to distinguish PK activities that are virally encoded from those that are induced in the host cells (36), the number, origin, and substrate specificities of PKs responsible for various herpesvirus-induced phosphorylations are not known. Only one herpesvirus PK has been identified, that encoded by the US3 open reading frame of herpes simplex virus (HSV). HSV gene US3 and the homologous gene in another alphaherpesvirus, varicella-zoster virus (VZV; gene 66), were first identified as putative PK genes based on their amino acid sequence similarity to known eucaryotic and retrovirus PKs (23). Recent biochemical and genetic studies have demonstrated that a specific PK activity (called the virus-induced PK, ViPK) (28, 29) is encoded by HSV gene US3 and that this activity is not essential for virus growth in cell culture (11, 30; D. P. Leader and F. C. Purves, Trends Biochem. Sci. **13**:244–246, 1988). The only other herpesvirus for which a complete genomic sequence is known, the gammaherpesvirus Epstein-Barr virus (EBV), does not appear to contain a gene corresponding to these two homologous PK sequences in the HSV and VZV alphaherpesviruses (8, 22, 23). We describe here the identification of a new set of homologous PK-related genes in HSV, VZV, and EBV. HSV gene UL13 and the homologous open reading frames in VZV (gene 47) and EBV (gene BGLF4) were identified as PK-related genes by using amino acid sequence patterns that are diagnostic of the catalytic domains of PKs.

Amino acid sequence patterns for PKs were constructed based on regions of conserved sequences within the catalytic domains of PKs. Six conserved regions previously identified in PK sequences (13, 16–18, 32, 33; D. P. Leader, Nature [London] **333**:308, 1988) are shown as regions I to VI in Fig. 1. Regions I and II appear to be at the ATP-binding site (18, 37). Regions III and IV are similar to a pair of conserved regions in bacterial phosphotransferases and thus may also function in ATP binding (S. Brenner, Nature [London] **329**: 21, 1987). Sequences between regions IV and V constitute the major autophosphorylation domain of PKs (16, 18, 34) and may be near the catalytic site. The function of region VI is unknown. Using conserved residues in regions I and III, Barioch and Claverie have devised two "sequence signatures" for PKs (Table 1, patterns I and III.A; A. Barioch and J.-M. Claverie, Nature [London] **331**:22, 1988). While the two patterns were reported to exhibit both high sensitivity (the ability to detect true-positive matches) and high specificity (the ability to reject false-positive matches) (2, 39) in identifying PK sequences, it has recently been noted that both patterns are absent in at least two known PK sequences, *Saccharomyces cerevisiae nim1* and *Drosophila* casein kinase II (Leader, Nature [London]). We have constructed new sequence patterns that together match all previously identified PK sequences in two protein data bases and, in addition, match PK-related sequences in homologous HSV, VZV, and EBV open reading frames that have not been previously identified.

To generate a set of PK sequences that can be used to assess the sensitivity of various sequence patterns, we collected known PK sequences from two protein sequence data bases, the Protein Identification Resource data base (PIR; v.15, 6,795 sequences, 1,684,561 amino acids) (12) and the PSEQIP data base (v.4.0, 8,117 sequences, 2,130,642 amino acids) (5). Previously identified PK sequences were collected in two ways. First, the two data bases were searched for sequences that match the two PK patterns of Barioch and Claverie (Table 1, patterns I and III.A) by using a regular expression search program (GGREP; developed by D. V. Faulkner, Molecular Biology Computer Research Resource, 1987; GGREP uses the regular expression handler from GNU-Emacs [35]). Sequences matched by the patterns but not previously identified as PKs were excluded from the collection of known PK sequences (see below). Second, the title and comment fields of all sequences in the two data bases were searched for the term "kinase," and sequences other than those noted as PKs were excluded from the PK collection. This identified 66 and 81 PK sequences in the PIR and PSEQIP data bases, respectively. All other sequences in the two data bases were considered apparent false-positive matches (apparent since further analysis may show them to be true PK sequences; e.g., as with the newly identified PK-related sequences in herpesviruses described here). Since the two data bases (and subsequently the two sets of PK sequences) comprise overlapping and noninclusive sets of sequences that could not be readily merged into a single set, the data bases were treated separately when the sensitivity and specificity of sequence patterns were determined.

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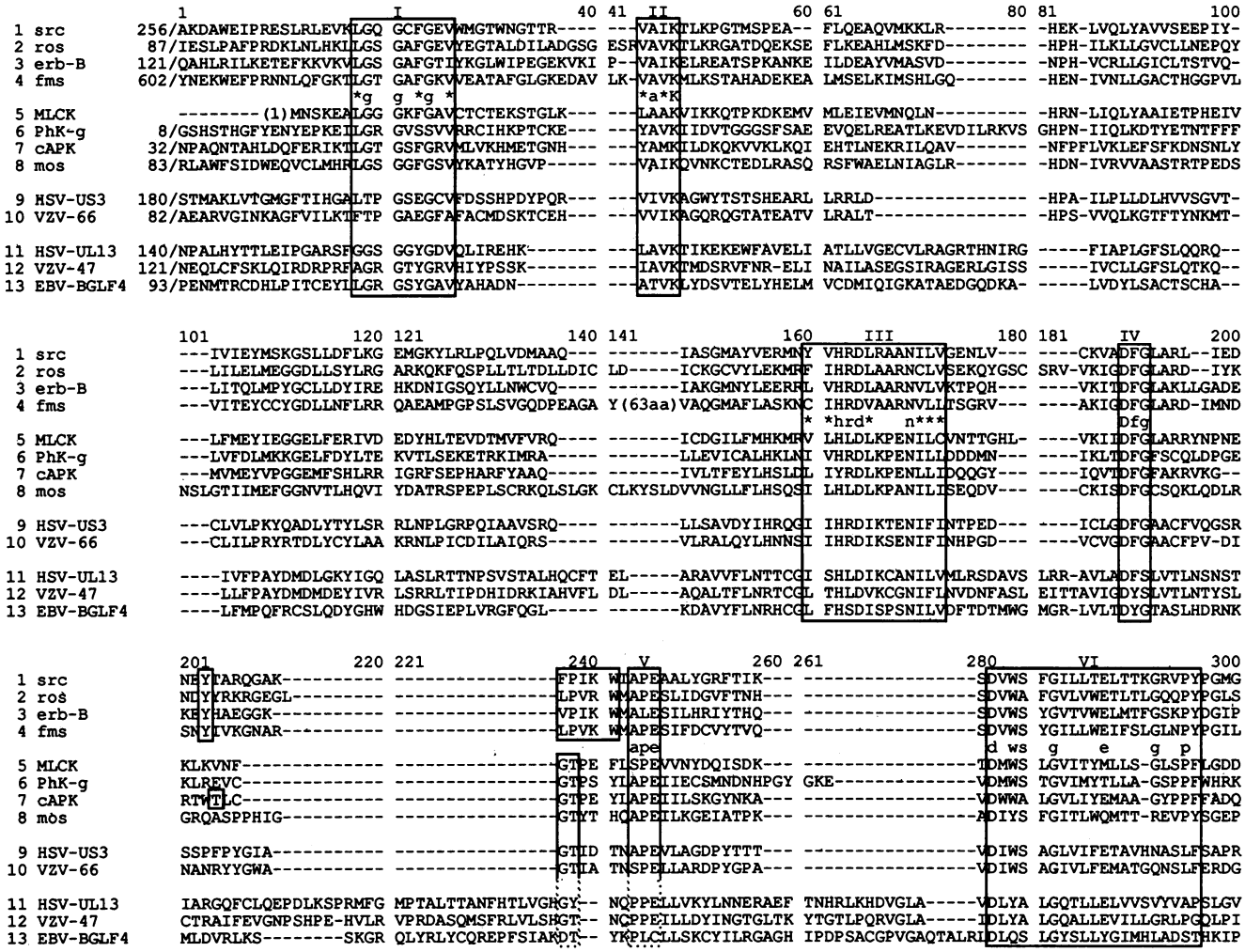

FIG. 1. Amino acid sequence alignments of the catalytic domains of PKs. Rows 1 to 4, Representative tyrosine kinases: Rous sarcoma virus pp60$^{v\text{-}src}$ (src; PSEQIP locus KSRC$RSVSR), avian sarcoma virus v-*ros* (ros; PSEQIP locus KROS$AVISU), avian erythroblastosis virus v-*erb-B* (erb-B; PSEQIP locus KERB$AVIER), and feline sarcoma virus v-*fms* (fms; PIR locus TVMVMD) (a 63-amino-acid region specific to this sequence [at rp 142; 18] has been omitted to conserve space). Rows 5 to 8, Representative serine/threonine kinases: rabbit myosin light chain kinase (MLCK; PIR locus A05120), rabbit phosphorylase *b* kinase gamma chain (PhK-g; PSEQIP locus KPBG$RABIT), bovine cyclic AMP-dependent PK (cAPK; PSEQIP locus KAPA$BOVIN), and Moloney murine sarcoma virus v-*mos* (mos; PSEQIP locus KMOS$MSVMO). Rows 9 and 10, Homologous serine/threonine-related PKs previously identified in herpesviruses (23): HSV type 1 open reading frame US3 (HSV-US3; PSEQIP locus KRTP$HSV1) and VZV gene 66 (VZV-66; PSEQIP locus VAZUSP2). Rows 11 to 13, Homologous herpesvirus sequences encoding putative serine/threonine PKs: HSV type 1 open reading frame UL13 (HSV-UL13; PSEQIP locus V57$HSV1), VZV gene 47 (VZV-47; translated from GenBank locus VAZXX), and EBV open reading frame BGLF4 (EBV-BGLF4; PSEQIP locus VIRU107). The sequences were aligned by eye based on the PK sequence alignment of Hunter and Cooper (18). Highly conserved regions in eucaryotic and retrovirus PKs (16–18, 21, 32, 33) are shown as boxed regions I to VI. Residues totally conserved in our collection of PK sequences are shown as capital letters beneath sequence 4; highly conserved residues are shown as lowercase letters; conservative amino acid groups are indicated by asterisks. The tyrosine (Y) residues autophosphorylated in retrovirus tyrosine kinases (17, 18) are shown boxed at rp 203 in sequences 1 to 4; the threonine (T) autophosphorylated in cyclic AMP-dependent PK (34) is shown boxed at rp 204 in sequence 7. Sequences conserved in tyrosine kinases (Table 1, pattern IV-V.Y) are shown boxed at rp 237 to 241 in sequences 1 to 4. Sequences conserved in serine/threonine kinases (Table 1, pattern IV-V.S/T) are shown boxed at rp 237 to 238 in sequences 5 to 10; potentially related residues in sequences 11 to 13 are shown as a dotted extension. Residues in sequences 11 to 13 potentially related to region V are shown as a dotted extension of region V.

Amino acid sequence patterns for region III with increased sensitivity and specificity in identifying PK sequences were then constructed as follows. First, a set of regular expression patterns were constructed describing the positions of invariant amino acids, conservative amino acid groups, and inconstant (wild card) sites within region III. The initial patterns were modeled on the PK pattern of Barioch and Claverie for this region (Table 1, pattern III.A) and on the sequences observed within region III in the alignment of 8 protein-serine and 12 protein-tyrosine kinases made by Hunter and Cooper (18). Second, the sensitivity and specificity of these patterns were evaluated. By using our regular expression search program, sequences matching each pattern were identified in (i) the collection of 66 and 81 PK sequences identified in the PIR and PSEQIP data bases (yielding a list of the false-negative matches for each pattern)

TABLE 1. Amino acid sequence patterns matching conserved regions in the catalytic domains of PKs

Name[a]	Pattern[b]
I.[c]	[LIV]GxGx[FY]Gx[LIV]
III.A[c]	[LIV] [HY] x D[FILMVY]xxxNx[FILMV] [FILMV]
III.B	[FILMVYC] x [HY] x D[FILMVY]xxxNx[FILMVYC] [FILMVYC]
III.C	[HY] [RL]D[FILMVY]xxxNx[FILMVYC] [FILMVYC]
IV-V.Y[d]	D[FWY]G*{17,22} [FILMV]P [FILMV] [KR]Wx[AGSP] [ALP]E
IV-V.S/T[e]	D[FWY]G*{12,22} (Gx or x[ST]) x x x x[AGSP] [ALP]E

[a] The pattern name is based on the conserved region that is matched in Fig. 1.
[b] Amino acids enclosed in brackets signify alternatives for that position; x matches any single residue; *{n,m} matches any string with a minimum length of n and a maximum length of m. Blank spaces were inserted into patterns to align equivalent positions.
[c] From Barioch and Claverie, Nature (London).
[d] Tyrosine (Y) kinase pattern.
[e] Serine/threonine (S/T) kinase pattern.

and (ii) the collection of all other sequences in the two data bases (yielding a list of the apparent false-positive matches; Table 2). Third, the patterns were revised to maximize their sensitivity and specificity. If the pattern sensitivity was low (producing a large number of false-negative matches), the false-negative matches were examined to identify the mismatched positions in the pattern. The patterns were then generalized at these positions to allow the excluded PK sequences to be matched. Conversely, if the pattern specificity was low (producing a large number of false-positive matches), amino acid groups were made more restrictive and/or additional positions in the region were added to the pattern to exclude matches to non-PK sequences. The second and third steps were then repeated until no further gains in pattern sensitivity and specificity were achieved.

By using the method described above, two new sequence patterns for region III were devised (Table 1, patterns III.B and III.C). Both patterns had increased sensitivity (i.e., fewer false-positive matches) for PKs compared with the motifs of Barioch and Claverie (Table 2); patterns III.B and III.C each failed to match only two PK sequences, *Drosophila* insulin receptor and the avian sarcoma virus *mil* MH2 gene. The sequences of these two PKs, however, were matched by pattern I, demonstrating the potential increase in sensitivity that can be achieved by the combined use of patterns derived from different conserved regions within a protein sequence. The sequence of fission yeast *nim1*$^+$, which was not matched by either of the patterns of Barioch and Claverie (I. and III.A), was matched by patterns III.B and III.C. The sequence of *Drosophila* casein kinase II (which was not at the time in either of the two protein data bases) was also matched by the two patterns.

The apparent false-positive matches to pattern III.B included several bacterial phosphotransferases that have a conserved domain very similar to regions III and IV of PKs (Brenner, Nature [London]). In addition, pattern III.B matched two herpesvirus open reading frames, HSV gene UL13 and EBV gene BGLF4. HSV-UL13 and EBV-BGLF4 were also matched by patterns III.C and I., respectively.

The sequences of all apparent false-positive matches shown in Table 2 were visually inspected for the presence of the six conserved regions previously identified within the catalytic domains of PKs (Fig. 1). Only two of these matches, HSV-UL13 and EBV-BGLF4, were found to contain similar regions. HSV-UL13, EBV-BGLF4, and an open reading frame in VZV, gene 47, have been previously shown to be homologous; the position, size, orientation, and sequences of these genes and the genes in their surrounding vicinity are conserved among these three herpesviruses (7, 8, 22, 24). The amino acid sequence of VZV-47 was not in either of the two protein data bases and was thus missed by our search. An alignment of the predicted amino acid sequences of HSV-UL13, VZV-47, and EBV-BGLF4 with the sequences of representative tyrosine and serine/threonine PKs (18) and with the two previously identified herpesvirus PKs, HSV-US3 and VZV-66, is shown in Fig. 1. The HSV-UL13, VZV-47, and EBV-BGLF4 genes clearly encode PK-related sequences, sharing all but perhaps one of six conserved regions within the catalytic domains of known PKs.

The only region that is not well conserved in these three homologous herpesvirus sequences is that surrounding region V. This region contains the major autophosphorylation site in PKs, with sequence features that are diagnostic of tyrosine versus serine/threonine PKs (16, 18). In tyrosine kinases, the amino acids between regions IV and V contain a conserved tyrosine adjacent to acidic residues (shown as a boxed Y at reference position [rp] 203 in Fig. 1, sequences 1 to 4) (17, 18). This tyrosine is autophosphorylated in retrovirus tyrosine kinases (17, 18). In the catalytic subunit of cyclic AMP-dependent PK, a serine/threonine kinase, a threonine is autophosphorylated in the same region (shown as a boxed T at rp 204 in sequence 7) (34). By examining amino acid sequences of this region in our collection of known PKs, we found sequences immediately N terminal of region V that distinguish tyrosine from serine/threonine kinases (Fig. 1, boxed regions at rp 237). On the basis of the amino acid sequences of these regions we devised a diagnostic sequence pattern for each class (Table 1, tyrosine [Y] kinase pattern named IV-V.Y and serine/threonine [S/T] kinase pattern named IV-V.S/T). The two patterns cleanly separate known PK sequences into these two classes and together have excellent sensitivity and specificity for PKs (Table 2). The two previously identified herpesvirus PKs, HSV-US3 and VZV-66, contain the serine/threonine motif (Fig. 1), consistent with the biochemical observation that the HSV-US3-encoded protein kinase (ViPK) has serine/threonine kinase activity (28). One EBV sequence (EBV gene BBRF3) was matched by pattern IV-V.S/T, but this sequence contains none of the other conserved regions diagnostic of PKs. The one previously identified PK sequence that was missed by these patterns is the yeast gene *CDC7*. This sequence matches the serine/threonine PK pattern (and has the spacing between regions V and VI typical of other PKs) but has a very large insertion of about 80 amino acids just after region IV (27).

In the HSV-UL13, VZV-47, and EBV-BGLF4 sequences, the putative autophosphorylation domain (between regions IV and VI) is about 40 amino acids longer than the corresponding domain in other PKs. Although these three sequences do not match the serine/threonine kinase pattern (pattern IV-V.S/T), a region in the center of the putative

TABLE 2. False-negative and apparent false-positive matches to PK sequence patterns

Pattern[a]	False-negative PKs[b]	Apparent false-positive PKs[c]
	PIR/PSEQIP locus (PK name)	PIR/PSEQIP locus (protein name)
I.	A25962/-- (yeast *wee1*$^+$) KIRBFG/KPBG$RABIT,MUSPHKGP1 (phosphorylase *b* kinase) KIZPMN/-- (yeast *nim1*$^+$) --/ONCO31 (avian *src*-AS) TVFFS/KSRC$DROME (*Drosophila src*) --/YSCCDC7P1 (yeast *CDC7*) --/YSCSTE7P1 (yeast *STE7*) TVBE17/KRTP$HSV1 (HSV-US3) --/VAZUSP2 (VZV-66)	P9AD37/HEX9$ADEN7 (adenovirus hexon-associated protein IX) QQAG4T/YP4$AGRTU,TIPMS2P1,TIPCTP2 (*A. tumefaciens* hypothetical protein 4) --/VIRU107 (EBV-BGLF4)
III.A	A05120,A25830/-- (myosin light chain kinase) A24147/-- (*Drosophila* insulin receptor) KIZPMN/-- (yeast *nim1*$^+$) --/AC2MILP1 (avian *mil*-MH2) --/HUMPSKBP1 (human *psk*-C3)	--/STRHYGP1 (hygromycin B phosphotransferase) --/VIRU193 (adenovirus type 2 16.4K URF)
III.B	A24147/--[d] (*Drosophila* insulin receptor) --/AC2MILP1[e] (avian *mil*-MH2)	A24594,SAZQK1/MSAP$PLAFK,MSAP$PLAFW (major merozoite surface antigen) 5 bacterial phosphotransferases[f] WMBE71/V57$HSV1[g] (HSV-UL13) --/VIRU107 (EBV-BGLF4) --/--[h] (VZV-47)
III.C	A24147/--[d] (*Drosophila* insulin receptor) --/AC2MILP1[e] (avian *mil*-MH2)	VCPV19/-- (parvovirus coat protein VP1) WMBE71/V57$HSV1[g] (HSV-UL13) --/--[h] (VZV-47)
IV-V.Y and IV-V.S/T	--/YSCCDC7P1[i] (yeast *CDC7*)	A24727 (phenylalanine ammonia-lyase) QQBE35/YBR3$EPBAR (EBV-BBRF3) YRNC/TTYNC,TYRO$NEUCR (*Neurospora* tyrosinase) --/YSCMFA2GP1 (yeast alpha-factor-2)

[a] From Table 1.
[b] Known PKs that are not matched by the pattern. Some of the PK sequences are not full-length and were not counted as false-negatives if the region of sequence that would be matched by a pattern is missing.
[c] Sequences in the data bases that are not in the sets of known PKs but that are matched by the pattern. HSV-UL13, VZV-47, and EBV-BGLF4 are counted as apparent false-positives since they were previously unidentified sequences.
[d] Single amino acid variation in pattern: [HY][RL]P.. etc.
[e] Single amino acid variation in pattern: [HY][RL]D[FILMVY]xxxS.. etc.
[f] PIR loci PKBSK, PKECT9, PKSMR, and PKSOJF; PSEQIP locus STRHYGP1.
[g] PSEQIP locus THV50 was also matched; this entry contains amino acid sequences from both HSV-UL13 and the adjacent open reading frame, UL14 (22), and was derived from a preliminary DNA sequence of this region (6, 38) that apparently contained frameshift errors.
[h] The sequence of VZV-47 is not in either data base but is matched by the pattern.
[i] Spacing variation: DFG.*{105}GTxxxxAPE.

autophosphorylation domain does resemble the sequences surrounding region V in serine/threonine-like kinases. The HSV-UL13 and VZV-47 sequences contain a three-residue sequence matching region V (EBV-BGLF4 has a single-residue mismatch), and all the herpesvirus sequences match an adjacent (Gx or x[ST]) pattern that is two, rather than four, residues N terminal from this region. Thus, while the three herpesvirus sequences do not exactly match the prototypic serine/threonine kinase pattern, they do contain sequences that suggest that they are serine/threonine-like kinases.

The best candidate for a PK encoded by the three herpesvirus sequences is a capsid-tegument-associated PK that has been found in a number of herpesviruses (10, 21, 31, 36). Except for the equine herpesvirus kinase (31), capsid-tegument-associated PKs phosphorylate virion polypeptides (VPs), but not exogenous substrates, and are biochemically distinct from the HSV-US3-encoded ViPK (20, 28, 29). Of the VPs phosphorylated by the capsid-tegument-associated PK activity, only one, VP 18.8, is not detected in amino-acid-labeled preparations of purified virions (21). Since a PK would be expected to be (auto)phosphorylated (9, 18) and in low abundance compared with capsid and tegument structural proteins, as is observed for VP 18.8, this protein may be the capsid-tegument-associated PK itself. VP 18.8 has been mapped by intertypic recombination to the same region as that of UL13 (between map units 0.15 and 0.18 for VP 18.8 and between 0.17 and 0.19 for UL13, on the physical map of HSV) (21, 22, 24). In addition, the predicted molecular mass of UL13 (57 kilodaltons [kDa]) is close to the observed size of VP 18.8 (approximately 55 kDa; although this is also similar to the molecular mass of 51 kDa predicted for another open reading frame in this region, UL10) (22, 24). Evidence relating the capsid-tegument-associated PK, VP18.8, and UL13 is at present circumstantial but could be tested by using the same biochemical and genetic techniques used to identify the PK activity (ViPK) and protein species (68 kDa) encoded by HSV-US3 (11, 30).

To identify other herpesvirus sequences related to PKs (i.e., those in open reading frames not currently available in

the protein data bases), the predicted amino acid sequences of all open reading frames identified in the complete genomic sequences of HSV, VZV, and EBV (1, 4, 7, 22) were searched with the PK patterns. No additional matches were observed. PK-related sequences might remain undetected, however, if they contain sequences that are less well conserved than those of the current set of identified PK sequences. We are currently testing additional patterns (e.g., based on conserved sequences in region VI) to identify other potential PK-related sequences in herpesviruses.

As far as we can determine, there are no other sequences in the protein data bases that are similar to the three newly identified PK-related sequences. The three herpesvirus sequences were compared with all sequences in the two protein data bases by using a high-speed similarity search program (DASHER; D. V. Faulkner and T. F. Smith, Molecular Biology Computer Research Resource, 1987; this program uses a modified Wilbur-Lipman algorithm [40]). This search yielded no other closely related matches, even with other known PK sequences. The identification of these PK-related sequences in herpesvirus open reading frames by using amino acid sequence motifs demonstrates the utility of this technique in identifying domains in functionally related sequences with little overall sequence similarity (15).

Shortly after submission of this paper, Hanks et al. (14) reported the identification of 11 conserved regions in the catalytic domains of PKs on the basis of an alignment of 38 protein-serine and 27 protein-tyrosine kinases. The HSV, VZV, and EBV PK-related sequences which we have identified display a one-to-one correspondence to these 11 regions: regions I to IX of Hanks et al. correspond to our regions I, II, rp 57 to 58, rp 86, rp 112 to 113, and regions III, IV, V, and VI, respectively, in the herpesvirus sequences (Fig. 1). The analysis of Hanks et al. also lends additional support to our tentative identification of the herpesvirus sequences as encoding serine/threonine kinases rather than tyrosine kinases. These investigators identified two regions within the catalytic domains of PKs that distinguish serine/threonine from tyrosine PKs. One is the region just N terminal of region V (near rp 240 of Fig. 1) that we used in patterns IV-V.S/T and IV-V.Y. The second distinguishing site is at rp 166 within region III. At this position, serine/threonine PKs have an invariant lysine (K) while tyrosine PKs have either an alanine (A) or an arginine (R) residue. HSV-UL13 and VZV-47, as well as the two previously identified herpesvirus PKs, HSV-US3 and VZV-66, have a lysine at this position. EBV-BGLF4 has a serine at this site, but this sequence appears to be more highly diverged than any of the PK sequences observed to date.

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