Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 2658–2661, April 1988
Evolution

Sequence analyses of herpesviral enzymes suggest an ancient origin for human sexual behavior

(primate sexual behavior/human and herpesviral evolution)

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Communicated by Wolfgang K. Joklik, December 21, 1987

ABSTRACT **Comparison of the amino acid sequences of the deoxythymidine kinases of herpes simplex (HSV) and of marmoset herpes viruses (MHV) suggests a divergence time of 8 to 10 million years ago for HSV-1 and -2. Like MHV, HSV-1 and -2 cause local infections in their natural hosts, and direct contact between two individuals during the brief period of infectivity is needed for transmission. Because B virus, a nearer relative of HSV, depends on both oral and genital routes of transmission, we postulate that ancestral HSV (aHSV) was similar, and that for HSV-1 and -2 to diverge, genital and oral sites had to become microbiologically somewhat isolated from each other, while oral–oral and genital–genital contact had to be facilitated to maintain both aHSV strains. We propose that acquisition of continual sexual attractiveness by the ancestral human female and the adoption of close face-to-face mating, two hallmarks of human sexual behavior, provided the conditions for the divergence.**

Several diverse findings published in the past 2 decades now allow an estimate for the time of evolutionary divergence of herpes simplex virus (HSV) types 1 (oral) and 2 (genital). These include methods for determining phylogenetic trees from differences in amino acid sequences of related proteins from different species (1, 2); the amino acid sequences of the enzyme deoxythymidine kinase (dTK) from several herpesviruses, HSV-1 and -2, and marmoset herpes virus (MHV) in particular (3–5); the documentation of the respective biological preferences of HSV-1 and -2 for oral and genital sites as well as the behaviors associated with their transmission (6); estimates for the time of divergence of Old and New World monkeys (7–10); and an understanding of the power and limitations of molecular biological methods in describing primate evolution (11, 12). In addition, knowledge of the reproductive behaviors of nonhuman primates (13) considered together with these findings suggests an approximate time of 8 to 10 million years (Myr) ago for the appearance of continual female sexual attractiveness,¶ and for the adoption of close face-to-face mating, two highly characteristic features of human sexual behavior.‖

In addition to the dTK sequences from HSV-1, HSV-2, and MHV, the following were also analyzed: dTK, from varicella zoster virus (VZV) and Epstein–Barr virus (EBV) (16–18); ribonucleotide reductase large subunit (RRL) from HSV-1, HSV-2, VZV, EBV, mouse, and *Escherichia coli* (16, 17, 19–22); ribonucleotide reductase small subunit (RRS) from HSV-1, HSV-2, VZV, mouse, clam (*Spisula*), *E. coli*, and T4 coliphage (16, 17, 22–29) and DNA polymerase (pol) from HSV-1 strain 17 (30), KOS (31), and Angelotti (32); HSV-2 (33), VZV (16), EBV (17, 30), human cytomegalovirus (CMV) (34) and vaccinia virus (35). Accepted point mutations per 100 residues (PAMs) for each possible pair within each of the four sequence groups were determined as described by Dayhoff *et al.*, using alignments done with the program ALIGN (36) of the Protein Identification Resource of the National Biomedical Research Foundation and the 250 PAM matrix (37), with conversion of percentage differences to PAMs as described (38).

Four trees (Fig. 1) were deduced for the four sets of sequences by the procedures described by Fitch and Margoliash (1) assisted by computing resources available in the PROPHET system.** The percentage standard deviations were 4.9, 5.1, 4.7, and 4.9 for the dTK, RRL, RRS, and pol trees, respectively, which compare favorably with those reported by Fitch and Margoliash for their cytochrome-based trees (1).

The dTK tree was calibrated by using the time of divergence of New and Old World monkeys and is based on the assumption that MHV and ancestral HSV (aHSV, later to become HSV-1 and HSV-2) diverged at about the same time as their respective hosts. We consider it unlikely that aHSV and MHV would have diverged significantly earlier than their hosts because they occupy similar niches in their respective hosts (39); a divergence of the two viruses later than the host divergence would require one of the hosts to lose the virus in the process and reacquire it later through an interspecific transfer, which seems equally unlikely.

The use of the time of divergence of New and Old World monkeys to calibrate the trees is complicated by the lack of general agreement on what that time was. Many recently published estimates are in the range of 35 to 55 Myr ago (7–10). Dates based on use of the so-called molecular clock tend to be more recent than others, as is the case with the time of divergence of humans and the great apes, now thought to be in the range of 4 to 8 Myr ago (40–43). Because we consider it unlikely that HSV-1 and HSV-2 began to diverge much before humans and the great apes (see below), we used the more recent estimate of 35 Myr ago [inferred from Fleagle *et al.* (10)] for calibration of the tree. This gives a value of 9.0 Myr ago for the divergence of HSV-1 and HSV-2, which is not far outside the range of estimates cited above. Because the MHV dTK was the only sequence of a nonhuman primate herpes virus enzyme available, we calculated the divergence time for VZV from the dTK tree and

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Abbreviations: HSV, herpes simplex virus; dTK, deoxythymidine kinase; MHV, marmoset herpes viruses; Myr, million years; VZV, varicella zoster virus; EBV, Epstein–Barr virus; RRL, ribonucleotide reductase large subunit; RRS, ribonucleotide reductase small subunit; pol, DNA polymerase; CMV, cytomegalovirus; PAM, accepted point mutation; aHSV, ancestral HSV.

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¶We prefer "attractiveness" to "receptivity" or "proceptivity"; as pointed out by Dahl (14) great apes usually permit mating under the right circumstances regardless of estrus status; attractiveness plus female selection (15, 69) thus controls most mating.

‖We do not imply that human mating is exclusively face-to-face.

**PROPHET National Computer Resource (Bolt, Beranek, and Newman Laboratories, Cambridge, MA).

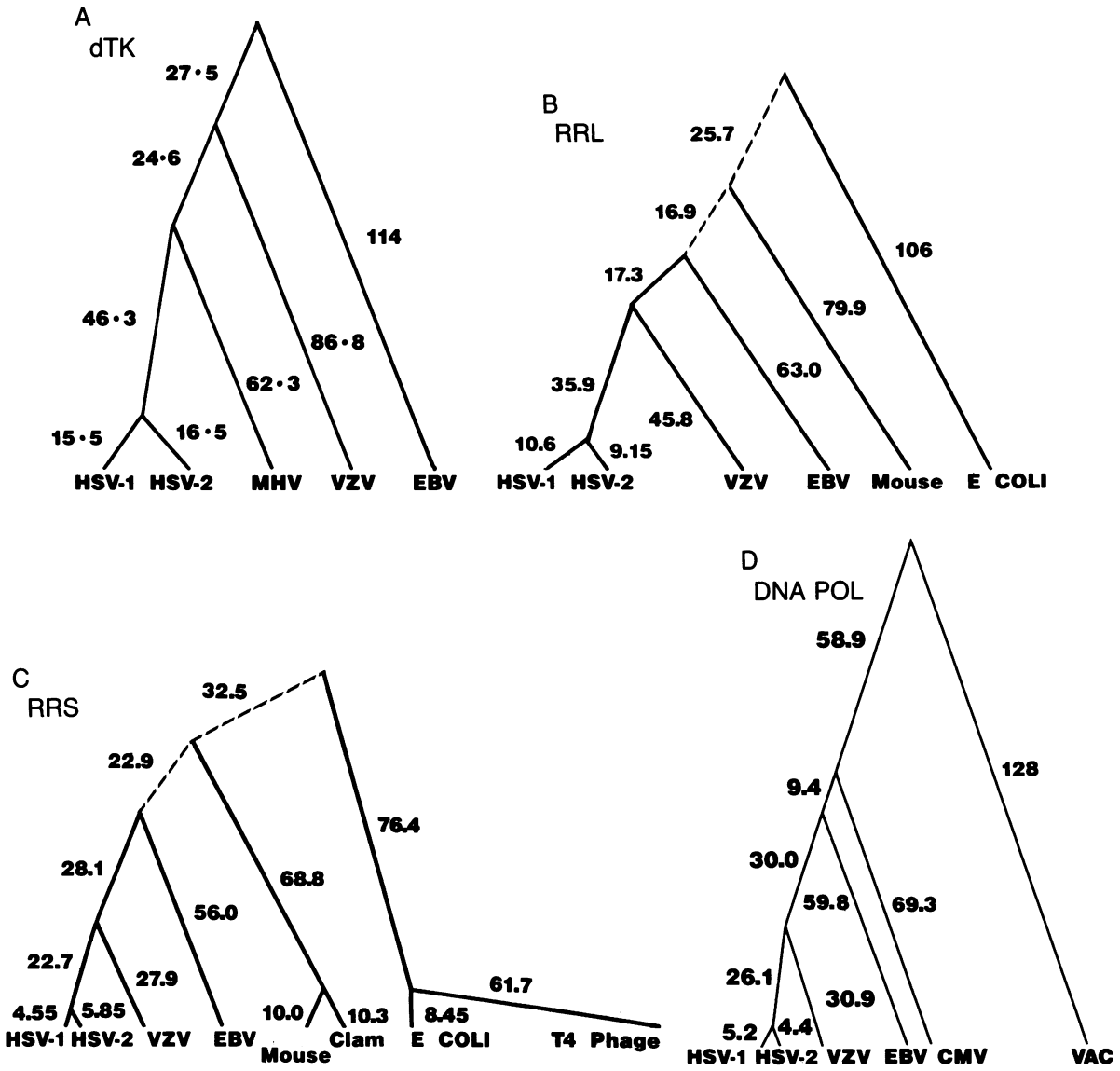

FIG. 1. Phylogenetic trees were constructed as indicated in the text. The values by each branch are in PAMs, accepted point mutation per 100 amino acid residues. HSV, human HSV; VAC, vaccinia virus; VZV, human VZV; EBV, human EBV; mouse, *Mus musculus*; clam, *Spisula solidissima*; T4, coliphage T4. Dashed lines (*B* and *C*) indicate a major discrepancy in the clock rate and imply that times calculated for the viral portions of the trees cannot be used to time events in the other portions.

used that value (48.6 Myr ago) to calibrate the other trees. When this was done, the divergence times for HSV-1 and HSV-2 as deduced from the RRS tree (9.0 Myr ago), the RRL tree (10.7 Myr ago), and the pol tree (7.5 Myr ago) were remarkably similar. The relative closeness of these four values lends support to the use of these trees to time events in herpesvirus evolution.

The usefulness of these trees in timing events, however, probably does not extend beyond the herpesviruses: from the RRS tree a value of only 17.7 Myr ago would be obtained for the divergence of clam and mouse, which is clearly absurd, and the evolutionary distance of vaccinia on the pol tree is large enough—relative to the herpesviruses—to cast doubt on its use in timing. Finally, Meyer *et al.* (44) have recently suggested that sequence comparisons such as those used here are unsuitable for timing events between prokaryotes and eukaryotes. The composite tree (Fig. 2), therefore, is limited to the herpesviruses only. From this tree it appears that CMV diverged from the common ancestor of EBV and VZV/HSV about 10 Myr before EBV and VZV/HSV diverged. This fits with the apparent tendency to localize with subsequent branching, as CMV (and presumably its common ancestor with EBV and VZV/HSV) typically affects a greater variety of tissues than any of the other viruses shown in the tree.

It is of interest to calculate the relative mutability of the different enzymes within the herpesviruses. From Table 1 it can be seen that the herpesviral dTK is highly mutable as compared to the RRS, RRL, and pol; all four are significantly more mutable than proteins from cellular sources. This may reflect the difference in generation time between viruses and cellular life; although generation time differences among metazooans were earlier not thought to account for apparent differences in molecular clock rates (46), we suspect that those arguments may not apply to viruses. Indeed, the recent report by Li and Tanimura (47) documents generation time differences even between hominoids. We suspect the unequal lengths between *E. coli* and T4 phage (Fig. 1*C*) reflect such a difference; similar effects have also been reported for pox (48) and papova (49) viruses. Furthermore, at least the HSV dTK is in itself highly mutable, as evidenced by the occurrence in populations of HSV virions of numerous mutant enzyme genotypes, which can be readily selected for (50, 51).

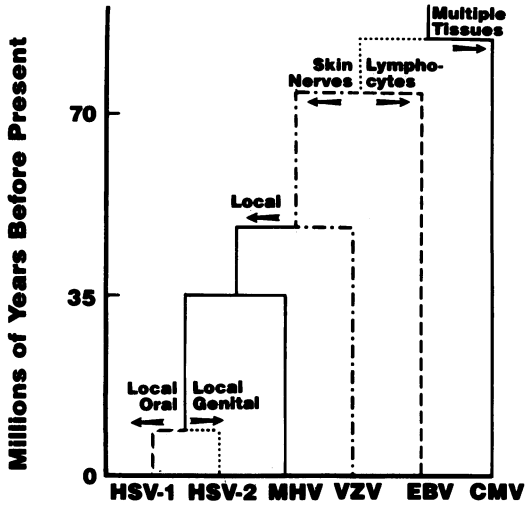

FIG. 2. Composite phylogenetic tree showing the evolution of the herpesviruses. Branch values are graphed on the *y* axis; the branch leading to MHV was arbitrarily set at 35 Myr ago (from the dTK tree; see text). The VZV branch (for the dTK tree) was then calculated to be 48.6 Myr ago and this value was used to calibrate the other trees. The various corresponding branches were then averaged to produce the composite tree. Important changes in characteristic pathogeneses are indicated at appropriate branch points.

The most interesting questions raised by this work are when, relative to the evolution of humans, did HSV-1 and HSV-2 diverge and why? Several factors are pertinent to these questions. First, B virus (*Herpesvirus simiae*), an HSV-like virus of Old World monkeys, appears to be transmitted both orally as well as genitally (52, 53), which suggests that oral and genital sites in the host are not microbiologically isolated. We assume that aHSV was similarly transmitted. Behaviors observed in modern great apes that would tend to mix the microbial flora of the two sites include frequent male–female indirect oral–genital contact (vagina to finger to mouth) as well as self-inspection and indirect oral grooming of the genitals following mating in *Pan troglodytes* (common chimpanzee) (ref. 54; C. E. G. Tutin, personal communication), oral stimulation of adult males by proceptive adolescent females in *Pongo* (orangutans) (55), and manual and oral inspection by juveniles of the genitalia of estrus females in *Gorilla* (56). Furthermore, autofellatio as well as female–male fellatio has been observed in captive *P. troglodytes* (J. F. Dahl, personal communication), although its frequency in the wild has not been determined. We assume that our common ancestors possessed similar behaviors. Second, although all the great apes limit almost all mating to maximum estrus (14), the length of estrus varies from species to species. In *P. troglodytes* it averages 9.6 days (54), sufficient time for a primary HSV-like infection to be acquired from one male and transmitted to another, while in *Gorilla* it is so short—2 days (57)—as to preclude this mode of transmission. *Pongo* (5.4 days) is intermediate in this respect (55). We assume that in this regard our common ancestor was also intermediate and that maintenance of aHSV in the species would depend sometimes on oral and sometimes on genital routes of transmission, with latency and recurrence playing the same role as in modern HSV. Third, although *P. troglodytes* and *Gorilla* both typically mate dorsal–ventral, *Pongo*, like *Homo*, mates ventral–ventral (55), while in *Pan paniscus* a significant fraction of matings are ventral–ventral (58, 59). In this regard, we assume that our common ancestor was like *P. troglodytes* and *Gorilla*; dorsal–ventral mating is the rule rather than the exception among other primates. Two conditions would appear to be required for the divergence of HSV-1 and -2. First, oral and genital sites would need to become increasingly microbiologically isolated, to minimize repeated mixing of the two diverging strains of aHSV. Second, frequent oral–oral, as well as genital–genital, contact would be needed for maintenance of the two evolving strains in their two respective sites. These conditions are consistent with Gause's observation (60) that competing species do not occupy the same ecological niche indefinitely. A generally upright posture resulting from bipedalism would seem to promote the isolation of genital and oral sites, possibly by making self-inspection and oral self-stimulation of the genitalia progressively more difficult and by keeping the face of one individual at a greater vertical distance from the genitals of another. A related and probably more pertinent consequence is the adoption of close face-to-face ventral–ventral mating; in the apes, ventral–ventral mating does not necessarily bring the faces into close proximity (61–62), although that possibility does exist in the pygmy chimpanzee (58). The requirement of frequent oral–oral and genital–genital contact could easily be accounted for by the combination of the extension of female sexual attractiveness through the entire menstrual cycle, with face-to-face mating, which, though driven primarily by the genital component of the behavior, would secondarily provide increased opportunity for oral–oral contact. It should also be noted that oral–oral contact does occur independently in *P. troglodytes*, primarily in the form of kissing after quarrels (63). Finally, we assume that the social system of our common ancestors more closely resembled those of *P. troglodytes* and *P. paniscus* than of *Pongo* or *Gorilla*, because of the more frequent interpersonal interactions in these systems (64, 65), which would promote the continued maintenance in the population of an HSV-like virus, with its relatively infrequent and brief periods of infectivity.

Table 1. Relative mutability of enzymes

Protein	PAMs per 10^8 yr
Herpesvirus dTK	178*
Herpesvirus RRL	94*
Herpesvirus pol	65*
Herpesvirus RRS	57*
Immunoglobulin κ chain constant region	37
Pancreatic ribonuclease	21
Phospholipase A_2	19
Carbonic anhydrase C	16
Animal lysozyme	9.8
Mammalian dTK	9.6*
Adenylate kinase	3.2
Triosephosphate isomerase	2.8
Animal RRS	1.5*
Glutamate dehydrogenase	0.9
Histone H4	0.1

*This project (data not shown for mammalian dTK); other values are from Dayhoff *et al.* (45).

The determinants of specific mammalian reproductive behaviors can be quite complex, and although a more detailed discussion of their origins and transmission is beyond the scope of this report, it has been suggested before that human reproductive behaviors are ancient and unique (66, 67). Furthermore, the proposal that behavior is an important driving force in vertebrate evolution (68) is consistent with our hypothesis. In summary, then, we propose that at about the time humans and apes diverged, the earliest human ancestors began to adopt an upright posture and, as a result, face-to-face mating. At about the same time, female sexual attractiveness began to extend over the entire menstrual cycle, with an attendant increase in frequency of

mating. These events, which are hallmarks of human sexual behavior, also began the divergence of HSV-1 and -2.

We thank L. Devine, A. Green, N. Lawrence, and M. Minyard for their expert assistance in the production of this manuscript, and the staff of the Rowland Medical Library for their cooperation. We also thank W. Barker, B. Clements, L. Corey, J. Dahl, J. Fleagle, L. Hunt, S. Kalter, B. MacFadden, H. M. McClure, R. D. Nadler, E. Simons, R. Susmann, and C. E. G. Tutin for their many helpful suggestions. This work was supported by National Institutes of Health Grant 2-S07-RR05386.

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