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Note

On the Mutation Rate of Herpes Simplex Virus Type 1

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ABSTRACT

All seven DNA-based microbes for which carefully established mutation rates and mutational spectra were previously available displayed a genomic mutation rate in the neighborhood of 0.003 per chromosome replication. The pathogenic mammalian DNA virus herpes simplex type 1 has an estimated genomic mutation rate compatible with that value.

ALL microbes with chromosomes composed of DNA, and for which both spontaneous mutation rates and mutational spectra have been determined, display a mutation rate in the laboratory of ~0.003 per genome replication, with a range of 0.0018–0.0046 and boundary values experimentally indistinguishable from 0.003 (Drake *et al.* 1998; Grogan *et al.* 2001). The DNA-based microbes that support this value are phage M13 with single-stranded DNA and a temperate mode of infection; phage λ with double-stranded DNA and alternating between lysogenic and lytic modes of infection; phages T2 and T4 with double-stranded DNA and a strictly lytic mode of infection; the eubacterium *Escherichia coli* growing optimally at ~37°; the archaeon *Sulfolobus acidocaldarius* growing optimally at ~80°; the yeast *Saccharomyces cerevisiae*; and the filamentous fungus *Neurospora crassa*. These organisms vary by nearly four orders of magnitude in genome size, and the average gene or base-pair mutation rate varies reciprocally by the same amount.

Another generality holds in most but not all DNA-based organisms: on average, the majority of mutations are base-pair substitutions (BPSs) (Grogan *et al.* 2001). The exceptions are thermophiles, which appear to have lower rates of BPSs than do mesophiles (Grogan *et al.* 2001; Friedman *et al.* 2004), perhaps because the average missense mutation is more deleterious at higher temperatures and therefore increases the intensity of selection for down-modifiers of the BPS mutation rate.

A major gap in the roster of microbial mutation rates has been a value for DNA viruses that infect animals. However, recently reported data can be used to fill this gap. The wild-type KOS strain of herpes simplex virus type 1 (HSV-1) cannot form plaques in the presence of various nucleoside analogs, while thymidine kinase (*tk*) mutants defective in the synthesis of thymidine kinase can do so. Ganciclovir is a guanosine analog that becomes strongly cytotoxic when phosphorylated. Using ganciclovir as the selective agent provided a HSV-1 tk^- mutant frequency of 6×10^{-5} and a mutation spectrum (Lu *et al.* 2002). To calculate a genomic mutation rate, this value must first be corrected for *tk* mutations that were not detected. A preferred way to do this (Drake 1991) is to assume that indels (insertions and deletions of base pairs) within the coding sequence are detected with high efficiency and that the total number of BPSs can be estimated from the number that produce internal stop codons (UAG, UGA, and UAA), which are usually null mutations; this is done by multiplying by 64/3 the number of chain-terminating codons produced by BPSs. In the present experiment, the mutational spectrum comprised 67 mutations of which 45 were indels and 5 were chain-terminating BPSs; this proportion (5/22 = 23%) of BPSs that produce chain-terminating codons falls in the center of the range (5–50%, average 20%) found for DNA-based microbes (Grogan *et al.* 2001). The corrected tk^- mutation frequency is thus $f_{tk} \approx 6 \times 10^{-5}[45 + 5(64/3)]/67 = 1.36 \times 10^{-4}$. [Alternatively, one could adjust the observed number of BPSs (22) by the average correction factor C = (total BPSs)/(detected BPSs) = 4.726 from Grogan *et al.* (2001) to obtain $f_{tk} \approx 6 \times 10^{-5}[45 + 22 \times 4.726]/67 = 1.33 \times 10^{-4}$.]

The geometry of DNA replication in HSV-1 is not fully understood, but probably comprises a mixture of exponential and linear components. For purely exponential

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replication, the mutation rate $\mu_e = f/\ln(N\mu_e)$, where f is the mutation frequency and N is the population size (Drake 1991). For purely linear replication with two rounds per cycle of cell infection, the mutation rate $\mu_l = f/2c$, where c is the number of cycles of cell infection (Drake and Holland 1999). In the experiments of Lu *et al.* (2002), the fraction of HSV-1 DNA packaged into progeny virions was 0.25–0.5, stock volumes were 1.5–2 ml, viable progeny were (1–5) $\times 10^6$/ml, and the ratio of particles to plaque-forming units was 10–50; combining these values gives $N \approx (2\text{–}4)(1.5\text{–}2)[(1\text{–}5) \times 10^6](10\text{–}50) = (0.3\text{–}20) \times 10^8$. Thus, the *tk*$^-$ rate $\mu_e \approx (1.33\text{–}2.11) \times 10^{-5}$ mutations/*tk* gene/replication, for which we will use the mean value of 1.72×10^{-5}. The value of c in these experiments was ~3, so that the *tk*$^-$ mutation rate $\mu_l \approx 2.26 \times 10^{-5}$. In the absence of information about the balance between exponential and linear replication, we will take the mean of these two values, $\mu_{tk} = 1.99 \times 10^{-5}$. The *tk* ORF contains 1128 bp (McKnight 1980) and HSV-1 contains 152,260 bp (McGeoch *et al.* 1988). Thus, the genomic rate $\mu_g \approx 0.0027$ mutations/replication.

The proportion of BPSs in the spectrum, 22/67 = 33%, is low for a nonthermophilic DNA microbe. The corresponding proportions, tabulated in Grogan *et al.* (2001), average 69% over many spectra and contain only four values <50%: those for phage T4 *ac* (48.5%), *E. coli lacI* (41.6%), mouse *gpt* (47.2%), and mouse *hprt* (25.5%). These four values and the HVS-1 value were each derived from drug-resistance systems in which it seems likely that a small amount of residual function suffices to prevent selection. Alternatively, some aspect of the HSV-1 life history may render the average missense mutation more deleterious (as suggested above for thermophily), thus enhancing selection for reduced BPS mutation rates.

An earlier study reported a HSV-1 *tk*$^-$ mutant frequency of ~10^{-3} in the KOS strain (Hall and Almy 1982), which corresponds to a genomic mutation rate of 0.026, substantially higher than that calculated here. One hypothesis for this difference, based on a comparison between HSV-1 and phage T4 (Drake *et al.* 1998, p. 1671), is that the KOS strain used in 1982 carried a mutator mutation. The KOS strain used by Lu *et al.* (2002) is not a direct descendant of that used by Hall and Almy (1982), so that the putative mutator mutation would have either arisen in the line used by Hall and Almy or been lost in the line used by Lu *et al.* (2002), a feasible event given the reduced fitness of mutator mutants in a stable environment (Mao *et al.* 1997; Funchain *et al.* 2000). A perhaps more tenable hypothesis is that the selection applied in 1982 by Hall and Almy used 5-bromodeoxyuridine, a moderate to strong mutagen in numerous organisms. The results of mutagenicity tests of ganciclovir seem to be described only in secondary sources (Wutzler and Thust 2001; Physicians' Desk Reference 2005, p. 2869), which depict it as clastogenic, recombinagenic, and mutagenic in the mammalian L5178YTK assay, but not mutagenic in either the mammalian *hprt* assay or the Ames salmonella assay. The latter two score gene mutations whereas the L5178YTK assay also scores very large multigenic deletions. Thus, ganciclovir is probably at most weakly mutagenic within the context of the HSV-1 *tk*$^-$ selection protocol, especially at the concentration of 10 μM applied to infected Vero cells by Lu *et al.* (2002).

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LITERATURE CITED

Drake, J. W., 1991 A constant rate of spontaneous mutation in DNA-based microbes. Proc. Natl. Acad. Sci. USA **88:** 7160–7164.

Drake, J. W., and J. J. Holland, 1999 Mutation rates among lytic RNA viruses. Proc. Natl. Acad. Sci. USA **96:** 13910–13913.

Drake, J. W., B. Charlesworth, D. Charlesworth and J. F. Crow, 1998 Rates of spontaneous mutation. Genetics **148:** 1667–1686.

Friedman, R., J. W. Drake and A. L. Hughes, 2004 Genome-wide patterns of nucleotide substitution reveal stringent functional constraints on the protein sequences of thermophiles. Genetics **167:** 1507–1512.

Funchain, P., A. Yeung, J. L. Stewart, R. Lin, M. M. Slupska *et al.*, 2000 The consequences of growth of a mutator strain of *Escherichia coli* as measured by loss of function among multiple gene targets and loss of fitness. Genetics **154:** 959–970.

Grogan, D. W., G. T. Carver and J. W. Drake, 2001 Genetic fidelity under harsh conditions: analysis of spontaneous mutation in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*. Proc. Natl. Acad. Sci. USA **98:** 7928–7933.

Hall, J. D., and R. E. Almy, 1982 Evidence for control of herpes simplex virus mutagenesis by the viral DNA polymerase. Virology **116:** 535–543.

Lu, Q., Y. T. Hwang and C. B. C. Hwang, 2002 Mutation spectra of herpes simplex virus type 1 thymidine kinase mutants. J. Virol. **76:** 5822–5828.

Mao, E. F., L. Lane, J. Lee and J. H. Miller, 1997 Proliferation of mutators in a cell population. J. Bacteriol. **179:** 417–422.

McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame *et al.*, 1988 The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:** 1531–1574.

McKnight, S. L., 1980 The nucleotide sequence and transcript map of the herpes simplex virus thymidine kinase gene. Nucleic Acids Res. **8:** 5949–5964.

Physicians' Desk Reference, 2005 *Physicians' Desk Reference,* Ed. 59. Thomson PDR, Montvale, NJ.

Wutzler, P., and R. Thust, 2001 Genetic risks of antiviral nucleoside analogues—a survey. Antiviral Res. **49:** 55–74.

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