Evolution of the mutation rate

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SUPPLEMENTAL MATERIAL

I. General Procedures for Estimating the Base-substitutional Mutation Rate / Nucleotide Site.

The general approach to estimating mutation rates from data on reporter constructs follows the procedures of Drake (1991), with some modifications as applied in Lynch (2010). The mutation rate to single-site base-pair substitutions was computed as

$$u_{BS} = u_L \cdot f_T \cdot f_{BS} / (L \cdot f_L \cdot f_D)$$

where u_L is the detected per-locus mutation rate; f_T is the fraction of mutations found after sequencing the candidate locus; f_{BS} is the fraction of detected mutations due to base substitutions; L is the length of the coding region (in bases); f_L is the fraction of the length wherein mutations have detectable phenotypic effects; and f_D is the fraction of base-substitutional mutations that are detectable.

The detectability factor was calculated as

$$f_D = x(n_m + n_n) / n_n$$

where n_m and n_n denote the numbers of observed missense and nonsense mutations, and the factor x denotes the fraction of base substitutions at sense codons that are expected to lead to nonsense mutations. Generally, as in Drake (1991), x was assumed to equal 3/64 = 0.047, based on the idea that 3 of 64 codons are chain terminating (which ignores bias in the mutational spectrum) and the fact that only a fraction of mutations at sense codons have functional effects. [For humans, when x was obtained more directly in a gene-specific manner (Lynch 2010), the resultant values of x ranged from 0.027 to 0.057, with a mean of 0.040 (0.001), not greatly different from this simpler approximation]. Using this approach, f_D takes on extreme values of x and 1.0 when the fractions of observable base-substitution mutations creating nonsense mutations at a locus are 1.0 and x, respectively. Use of the scaling factor f_D assumes that all nonsense mutations within the region of the gene susceptible to phenotypic effects are detectable, and to the extent that this is not the case, the mutation rate will be underestimated. In addition, f_L is often unknown and must then be assumed to equal 1.0, which will cause an underestimate of the mutation rate if the true value of f_L is <1.0.

Many studies of mutation in microbes simply report the frequencies of mutations rather than the actual locus-specific rates (u_L). Using the approach of Drake (1991), if the frequencies were determined from exponentially growing cultures with a measure of final population size available, the per-locus mutation rates could be estimated from the rate of accumulation of mutations per population doubling. Otherwise, the data were deemed unusable for mutation-rate estimation purposes. Per-locus rates were converted to per-site rates after correcting for the number of susceptible sites and the detectability of mutations at such sites, along with the fraction of mutations that could actually be attributable to the target locus when possible. The latter scaling involved extrapolations from nontarget species in some cases. The reader should be aware that the estimates cited below have many sources of error, particular in the case of microbial species, where the estimates are based on reporter constructs, which in some cases have very small target sizes.

Specific details follow for each taxon reported on in Figure 1.

RNA Viruses:

Chrysanthemum chlorotic mottle viroid Gago et al. (2009) estimate the total mutation rate per site to be 2500000×10^{-9}. After correcting for the estimated sequencing error rate noted by the authors, and further noting that 4 of 15 observed mutations involved insertion/deletions, the rate for base-substitutions mutations is $u_{BS} = 1850000 \times 10^{-9}$.

ϕ6. $u_{BS} = 1461 \times 10^{-9}$. From an experiment involving nearly complete genome sequencing by Burch et al. (2007), after accounting for genome size and the fraction of total mutations involving single-base substitutions (52/53), an estimate of 222×10^{-9} is obtained. Chao et al. (2002) give an earlier (and much higher) estimate based on reversions of a single amber mutation, 2700×10^{-9}. The average of these two estimates is used here.

Poliovirus. $u_{BS} = 3920 \times 10^{-9}$. Obtained directly from the genome-wide estimate in Table 1 of Drake and Holland (1999), after dividing by the genome size and 1.462 to account for the estimated fraction of indel mutations given by the authors.

Measles virus. $u_{BS} = 3920 \times 10^{-9}$. Obtained directly from the genome-wide estimate in Table 1 of Drake and Holland (1999), after dividing by the genome size and 1.462 to account for the estimated fraction of indel mutations given by the authors.

Rhinovirus. $u_{BS} = 6430 \times 10^{-9}$. Obtained directly from the genome-wide estimate in Table 1 of Drake and Holland (1999), after dividing by the genome size and 1.462 to account for the estimated fraction of indel mutations given by the authors.

Vesicular stomatitis virus. $u_{BS} = 6800 \times 10^{-9}$. Obtained directly from the genome-wide estimate in Table 1 of Drake and Holland (1999), after dividing by the genome size and 1.462 to account for the estimated fraction of indel mutations given by the authors.

Tobacco mosaic virus. $u_{BS} = 603 \times 10^{-9}$. Obtained directly from the estimate provided by Malpica et al. (2002), after accounting for the fraction of expected base-substitutional mutations (52.6/76.6 after correcting for detectability of base substitutions).

Single-stranded DNA Viruses:

ΦX174. $u_{BS} = 1404 \times 10^{-9}$. Data taken directly from Cuevas et al. (2009), averaging their own rate estimate and those from other studies, all of which are in good agreement with each other.

Bacteriophage M13. $u_{BS} = 654.7 \times 10^{-9}$. Drake (1991) provides a total per-site mutation rate estimate for the LacZ locus. Of the observed mutations, 50 were indels, and another 117 were base substitutions, which scale up to an expected value of 479 assuming an average detectability of ~0.14 (an average from human data, Lynch 2010). The rate of base-substitutional mutation per site was then taken to be (479/529) times the total rate.

Double-stranded DNA Viruses and Phage:

Herpes simplex virus. $u_{BS} = 12.42 \times 10^{-9}$. Drake and Hwang (2005) provide a total per-locus mutation rate estimate for thymidine kinase, which is 1128 bp in length. The detected mutations included 45 indels,

5 nonsense, and 17 missense base substitutions. After applying a detectability of base substitutions of 0.206 and assuming that all indels are detectable (as done in the following taxa as well), an estimated number of 107 actual base substitutions is inferred, and the per-site mutation rate was derived by scaling the per-locus rate by the locus size and the expected fraction of base substitutions.

Bacteriophage lambda. $u_{BS} = 58.40 \times 10^{-9}$. Drake (1991) provides a total per-site mutation rate estimate for the cI prophage suppressor gene. Of the observed mutations, 111 were indels, and 16 were nonsense and 4 were missense base substitutions. With a detectability of base substitutions at this locus of 0.059, 341 are expected to have actually occurred. The base-substitutional mutation rate was then estimated to be (341/452) times the total rate.

Bacteriophage T4. $u_{BS} = 14.51 \times 10^{-9}$. Drake (1991) provides a total per-site mutation rate estimate for the cI prophage suppressor gene. Of the observed mutations, 94 were indels, and after correcting for the detectability using a factor of 0.1125, 240 base substitutions were expected to have occurred. The base-substitutional mutation rate was then estimated to be (240/334) times the total rate.

Bacteriophage T2. $u_{BS} = 19.40 \times 10^{-9}$. Drake (1991) provides a total per-site mutation rate estimate and mutational spectrum data for the rII locus. The base-substitutional mutation rate was then estimated in the same manner as for bacteriophage T4.

Eubacteria:

Bacillus anthracis. $u_{BS} = 0.21 \times 10^{-9}$. The average per-locus mutation rate at the RpoB locus from three studies (Vogler et al. 2002; Zeibell et al. 2007; Yang and Miller 2008) is 1.79×10^{-9}, and the survey from these studies indicate that only 14 sites are involved. The method only detects base-substitution mutations, the detectability of which can be estimated from extensive work in *E. coli* that indicates 79 detectable mutation types over a total of 43 sites, yielding $79/(3 \times 43) = 0.612$ detectability (the three accounting for the three possible mutation types per site) (Wolff et al. 2004). [This estimate of detectability is used in other studies, cited below, that rely on the RpoB locus, and should be interpreted with caution]. The per-site rate is then obtained by dividing the per-locus rate by (14×0.612).

Bacillus subtilis. $u_{BS} = 0.74 \times 10^{-9}$. The average per-locus mutation rate at the RpoB locus from two studies (Nicholson and Maughan 2002; Perkins et al. 2008) is 6.32×10^{-9}. From these studies and Rossolillo and Albertini (2001), mutations have only been found at nine sites. The per-site mutation rate estimate was obtained using the same corrections as for *B. anthracis*.

Buchnera aphidicola. $u_{BS} = 4.00 \times 10^{-9}$. Rate taken directly from Moran et al. (2009).

Deinococcus radiodurans. $u_{BS} = 1.69 \times 10^{-9}$. The average per-locus mutation rate at the RpoB locus from two studies (Kim et al. 2004; Mennecier et al. 2004) is 22.75×10^{-9}, and from these studies 22 sites are known to exhibit detectable mutations. The per-site rate was estimated by assuming 22 sites and a detectability of 0.612 (as in *E. coli*).

Escherichia coli. $u_{BS} = 0.26 \times 10^{-9}$. Taken from the summary estimate for reporter genes in Lynch (2006).

Helicobacter pylori. $u_{BS} = 2.72 \times 10^{-9}$. Mutation-rate estimates for three loci were averaged. 1) The average per-locus mutation rate at the RpoB locus from two studies (Wang et al. 2001; Eutsey et al. 2007) is 7.65×10^{-9}, and assuming 43 sites and a detectability of 0.612 (as in *E. coli*), the estimated mutation rate is 0.29×10^{-9}. 2) The per-locus mutation rate at the GyrA locus) is 11.0×10^{-9} (Wang et al. 2001, and only two sites appear to yield mutations, each with 0.67 detectability. After noting that 9% of putative

mutations at this locus are not located there (Moore et al. 1995), the estimated mutation rate is 7.47×10^{-9}. 3) The per-locus mutation rate for clarithromycin resistance at the 23s rRNA is 0.80×10^{-9}, but the effective number of sites is only two, so the per-locus rate is 0.40×10^{-9}.

Mycobacterium fortuitum. $u_{BS} = 1.32 \times 10^{-9}$. Mutation-rate estimates were available from Gillespie et al. (2005) for RpoB and GyrA, which were corrected to per-site rates using the parameters noted above for *Helicobacter*, yielding 2.55×10^{-9} and 0.10×10^{-9}, respectively.

Mycobacterium tuberculosis. $u_{BS} = 0.19 \times 10^{-9}$. The average per-locus mutation rate at the RpoB locus from two studies (Werngren and Hoffner 2003; Mariam et al. 2004) is 2.13×10^{-9}, and the data from these studies and Morlock et al. (2000) suggest that only a fraction 0.66 of rifampicin-resistance mutations are actually found at the locus, and that only three codons are involved. Letting the number of sites be 12 and the detectability be 0.612 (as in *E. coli*), the estimated mutation rate is 0.29×10^{-9}.

Pseudomonas aeruginosa. $u_{BS} = 0.84 \times 10^{-9}$. The mutation rate per locus for FosA is 56.5×10^{-9} (Rodríguez-Rojas and Blázquez 2009). According to Beharry and Palzkill (2005), two-thirds of the amino acids of the 135 residue protein are essential, so assuming 25% silent sites (a good approximation for most organisms), the target size is $2/3 \times 3/4 \times 135 = 67.5$ nucleotides, and the rate per site is obtained by dividing the per-locus rate by this number.

Salmonella enterica. $u_{BS} = 0.17 \times 10^{-9}$. Mutation-rate estimates for two loci were averaged. 1) The average per-locus mutation rate at the RpoB locus from two studies (Hudson et al. 2002, 2003), one for rifampicin resistance and corrected assuming 43 sites and 0.612 detectability, and the other involving a single reversion and corrected by multiplying the per-locus rate by three to account for detectability at the site, 0.13×10^{-9}. 2) Hudson et al. (2002, 2003) give estimates for LacZ reversions, which requiring one specific base substitution, and multiplying the average of their per-locus rates by three to account for detectability yields a per-site rate of 0.21×10^{-9}.

Salmonella typhimurium. $u_{BS} = 0.15 \times 10^{-9}$. Data allowing three mutation-rate estimates are available in Hughes and Andersson (1997). 1) The average per-locus mutation rate at the RpoB locus from two studies (Hughes and Andersson 1997; Lind and Andersson 2008) is 2.47×10^{-9}, which after assuming 43 sites and 0.612 detectability yields a per-site rate of 0.09×10^{-9}. 2) From Hughes and Andersson (1997), the rate of origin of histidine auxotrophy is 0.5×10^{-6}, and Hartman et al. (1971) note that 67.3% of mutations are base substitutions. Of the base substitutions analyzed, 28 of 140 were nonsense, so the base- substitution detectability is 0.234. Using a total target size is 7389 bp from Drake (1991), the per-site rate is then 0.21×10^{-9}. 3) Hughes and Andersson (1997) also give a reversion rate for LacZ, which yields a per-site estimate of 2.4×10^{-9}. As this study is based on just one specific change at one site, and given the availability of more broadly sampling estimates, this estimate was not used in obtaining the final average.

Thermus thermophilus. $u_{BS} = 0.14 \times 10^{-9}$. Mackwan et al. (2008) selected for pyrimidine auxotrophs at the PyrE and PyrF loci, which together represent 1326 nucleotides of coding DNA. Here, the mutations had no detectable effects on fitness and no obvious phenotypic lag. Mutant sequencing indicates that 97.3% of detected mutants are actually represented at the locus. Correcting for this and averaging over two methods employed by the authors, the mutation rate per locus is 321×10^{-9}. With 24.7% of mutations being base substitutions, two of which were nonsense and 16 missense (implying a detectability of base substitutions of 0.422), the estimated per-site rate is $(321 \times 10^{-9}) \times 0.973 \times 0.247 / (1326 \times 0.422) = 0.14 \times 10^{-9}$.

Archaea:

Haloferax volcanii. $u_{BS} = 0.02 \times 10^{-9}$. Three per-locus mutation-rate estimates are available for the PyrE2 (Mackwan et al. 2007), but because mutations in this system exhibit phenotypic lag, only the higher estimate derived from a mutation-accumulation approach is used, 58.7×10^{-9}. Only 73.9% of mutations could be found molecularly at the locus, and of these 11.8% are base substitutions. The mutational target size is 531 bases, and assuming a detectability of 0.422 as in *T. thermophilus*, the base-substitutional mutation rate is estimated to be $(58.7 \times 10^{-9}) \times 0.739 \times 0.118 / (531 \times 0.422) = 0.023 \times 10^{-9}$. However, even this highest estimate likely a substantial estimate, as cells have a high ploidy level, which causes recessive mutations to be masked (Drake, pers. comm.), so the estimate for this species was not used.

Sulfolobus acidocaldarius. $u_{BS} = 0.05 \times 10^{-9}$. Jacobs and Grogan (1997) used 5-fluoro-orotic acid to select for pyrimidine auxotrophs at the PyrE and PyrF loci. FOA is not toxic, but the byproduct of action by these two genes is, so inactivation of this pathway eliminates toxicity. The aggregrate estimate used here is based on the mutation rate from this and a second study (Grogan et al. 2001), and was calculated by Drake (2009) specifically for base-substitution mutations.

Unicellular eukaryotes:

Neurospora crassa. $u_{BS} = 4.66 \times 10^{-9}$. Mutation-rate estimates for four loci were averaged. 1) Koh and Catchside (2007) provide an estimate for a single-site reversion to histidine utilization of 10.5×10^{-9}. 2) Three estimates of the per-locus mutation rate are available for the Mtr locus (methyl tryptophan resistance), which encodes a neutral amino-acid permease (Stadler et al. 1991). Scoring only uninucleate conidia (about one-fifth of the total), Chary et al. (1994) obtain an estimate of 3.0×10^{-7}; frequencies of detected mutations are given by Watters and Stadler (1995), which can be converted to mutation rates, and after multiplying by 5.0 to correct for undetectable multinucleates, yields an estimate of 7.0×10^{-7}; correcting for the fraction of undetectable multinucleates, the data of Dillon and Stadler (1994) yield an estimate of 2.0×10^{-7}. With an average per-locus rate of 400×10^{-9}, 32.4% of assayed mutations being base substitutions, a total coding sequence for the gene of 1413 bp, and employing a detectability of base substitutions of 0.143 (from the average of human genes (Lynch 2010), in the absence of the relevant information for *N. crassa*), the mutation rate per site is then $(400 \times 10^{-9}) \times 0.324 / (1413 \times 0.143) = 0.64 \times 10^{-9}$. 3) Dillon and Stadler (1994) give a reversion rate for one site in Trp-2 of 1.1×10^{-9}, which after multiplying by three, yields a per-site rate of 3.3×10^{-9}. 4) Two estimates are available for the per-locus rates at Ad-3a. Control data from Sakai et al. (2003) yield an estimate of 240×10^{-9}; frequency data from de Serres et al. (1999) summed over the Ad-3a and Ad-3b regions yield an estimate of 139×10^{-9}, after accounting for the fact that only 24% of point mutations are at Ad-3a. Averaging over these two rates, and noting that Ad-3a is 924 bp in length, that 82% of mutations appear to be point changes (de Serres et al. 1995), and using Drake's (1991) factor of 3.55 to correct for limited abilities to detect mutations in sectoring colonies and the average detectability factor of 0.143 for human base-substitutional mutations, the per-site rate is estimated to be $(190 \times 10^{-9}) \times 0.82 \times 3.55 / (924 \times 0.143) = 4.2 \times 10^{\wedge-9}$.

Plasmodium falciparum. $u_{BS} = 2.08 \times 10^{-9}$. Paget-McNicol and Saul (2001) measure the rate of origin of mutations at the DHFR (dihydrofolate reductase) locus by evaluating the response to pyrimethamine selection. The authors developed a modeling approach to estimate the mutation rate, and taking the average from two experiments yields an average per-locus rate of 1.390×10^{-9}. Just two specific mutations are known to confer resistance, a leucine to serine at codon 46 (TTA to TCA) and a serine to asparagine (AGC to AAC) at codon 108. Assuming a target size for base substitutions of two nucleotides, and 1/3 of the mutations at those sites being detectable, the per-site mutation rate is obtained by dividing the per-locus rate by 2/3.

Saccharomyces cerevisiae. $u_{BS} = 0.52 \times 10^{-9}$. A per-site estimate based on complete-genome of 0.33×10^{-9} is available from Lynch et al. (2008), and this was simply average with the prior average estimate from reporter-construct studies, 0.72×10^{-9}, obtained from analyses in Lynch (2006) and Lang and Murray

(2008).

Schizosaccharomyces pombe. $u_{BS} = 0.82 \times 10^{-9}$. Estimates are available for two loci. 1) Studies on Ade6-485 detect a reversion at a single stop codon (3 sites), with 5/9 possible mutations giving a functional revertant, so dividing the total reversion rate by 5/3 gives the rate per site. The average rate over three studies (Kunz and Fleck 2001; Tournier et al. 2001; Holmberg et al. 2005) is 1.35×10^{-9}. 2) The average per-locus rate for Ura4/5 over several studies (Rudolph et al. 1999; Chang et al. 2001; Kunz and Fleck 2001; Fraser et al. 2003) is 72.2×10^{-9}. From Rudolph et al. (1999) and Fraser et al. (2003), 79% of mutations are base substitutions, and from Genbank the total coding-region target size is 1437 bp, so using the average detectability for human base substitutions (0.143), the per-site rate is estimated as $(72.2 \times 10^{-9}) \times 0.79 / (1437 \times 0.143) = 0.28 \times 10^{-9}$.

Trypanosoma brucei. $u_{BS} = 1.65 \times 10^{-9}$. Valdes et al. (1996) estimated the rate of origin of loss of functional mutations in the Herpes simplex virus thymidine kinase gene inserted into the *T. brucei* genome. Averaging over four experiments, the per-locus mutation rate estimated from the frequency of nonmutant containing cultures and the number of cells per culture subject to mutation, is 532×10^{-9}. From Genbank, the open-reading frame is 1130 bp in length, and the authors sequenced four mutant alleles and obtained two frameshifts, one missense subsitution, and one nonsense substitution. Using the average human base-substitution detectability (0.143), the estimated per-site rate is then $(532 \times 10^{-9}) \times 0.5 / (0.143 \times 1130)$.

Multicellular eukaryotes:

Arabidopsis thaliana. $u_{BS} = 6.50 \times 10^{-9}$. Taken directly from a complete-genome sequencing project on mutation accumulation lines (Ossowski et al. 2009).

Caenorhabditis elegans. $u_{BS} = 5.75 \times 10^{-9}$. The average from two large projects, one involving complete-genome sequencing, evaluating mutation-accumulation lines (Denver et al. 2004, 2009).

Drosophila melanogaster. $u_{BS} = 4.65 \times 10^{-9}$. Taken directly from a complete-genome sequencing project on mutation accumulation lines Keightley et al. 2009).

Homo sapiens. $u_{BS} = 12.85 \times 10^{-9}$. Taken directly from a large-scale study inferring mutation rates indirectly from autosomal dominant and X-linked genes underlying monogenic human genetic disorders, and subjected to molecular surveys for causal mutations.

Mus domesticus. $u_{BS} = 36.99 \times 10^{-9}$. The estimate is the average derived from information from four types of studies. 1) Averaging over several visible markers and hundreds of thousands of observations, correcting for mosaics, and averaging over the sexes, data from Table 5 of Russell and Russell (1996) yield an average per-locus rate of 7080×10^{-9} per generation. From Mouse Genome Sequencing Consortium (2002), the average coding target size of these loci is 1320 bp. Assuming the average fraction of mutations that are base substitutions to be the same as in human monogenic disease-associated loci (0.504, from Lynch 2010) and an average detectability of 0.143, the per-site mutation rate is estimated as $(7080 \times 10^{-9}) \times 0.504 / (1320 \times 0.143) = 18.90 \times 10^{-9}$. 2) From Ono et al. (2000), the frequency of detectable mutations in the LacZ gene (transformed into mouse chromosomes) in the testes at the time of maturity is 71000×10^{-9}, and these numbers are quite constant throughout the lifespan of the mouse From Russell and Russell (1996), the ratio of female to male mutations is 25:42, so the sex-averaged rate is obtained by multiplying the male rate by $0.5[1+(25/42)] = 0.80$. From Ono et al. (2000), 98.6% 67.3% of these are independent (as opposed to being possible clonal expansions), and 85.4% of mutations are base substitutions, so assuming a coding region of 3075 bp and an average base-substitution detectability as in humans (0.143), the mutation rate per site per generation is estimated to be $(71000 \times 10^{-9}) \times 0.80 \times 0.986$

$\times$ 0.854 $\times$ 0.673 / (3075 $\times$ 0.143) = 73.20 $\times$ 10^{-9}. 3) Averaging over several studies (Kohler et al. 1991; Dycaico et al. 1994; Nishino et al. 1996; Walter et al. 1998; Hill et al. 2004, 2005), the frequency of detectable mutations in the LacI gene (transformed into mouse chromosomes) in the testes at the time of maturity is 11200 $\times$ 10^{-9}. From Walter et al. (2004) and de Boer et al. (1997), 96.5% of putative mutations are found when the locus is sequenced, and from Dycaico et al. (1996) 71.4% of mutations are base substitutions, so correcting for sex bias and possible clonality of mutations, assuming a coding region of 1094 bp an average base-substitution detectability as in humans (0.143), the mutation rate per site per generation is estimated to be (11200 $\times$ 10^{-9}) $\times$ 0.80 $\times$ 0.965 $\times$ 0.714 $\times$ 0.673 / (1094 $\times$ 0.143) = 26.56 $\times$ 10^{-9}. 4) Averaging over two studies (Masumura et al. 1999; Aoki et al. 2007), the frequency of detectable mutations in the Gpt gene (transformed into mouse chromosomes) in the testes at the time of maturity is 4250 $\times$ 10^{-9}. From several studies (Horiguchi et al. 1999; Masumura et al. 2000, 2003; Takeiri et al. 2003; Hashimoto et al. 2007; Ikeda et al. 2007; Yamauchi et al. 2008), 84.1% of mutations are base substitutions, so assuming all putative mutations reside at the locus, correcting for sex bias and possible clonality of mutations, assuming a coding region of 459 bp an average base-substitution detectability as in humans (0.143), the mutation rate per site per generation is estimated to be (4250 $\times$ 10^{-9}) $\times$ 0.80 $\times$ 1.00 $\times$ 0.841 $\times$ 0.673 / (459 $\times$ 0.143) = 29.32 $\times$ 10^{-9}.

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II. Rodent Somatic Mutation Rates.

Somatic mutation rates for the mouse reported in Table 1 and Figure 3 were obtained from observations on reporter constructs, using the same methods noted above for male germline mutation rates. Many of these estimates are derived from control data from experiments in environmental toxicology.

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