Heat Mutagenesis in Bacteriophage T4: Another Walk down the Transversion Pathway

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Extracellular nonrepeating bacteriophage T4 particles accumulate mutations as functions of temperature, time, pH, and ionic environment via two mechanisms: 5-hydroxymethylcytidine deamination produces G \( \rightarrow \) C \( \rightarrow \) A \( \rightarrow \) T transitions while a guanosine modification produces transversions. Neither frameshift mutations nor transversions at A T base pairs are appreciably induced. We now show that heat induces G C \( \rightarrow \) T A transversions which we suggest may arise via a G* A mispair, in which G* is a modified guanosine that has experienced a glycosidic bond migration. The rate of this reaction at 37°C is sufficient to present a genetic hazard, particularly to large genomes; thus, the lesion is probably efficiently repaired in cellular genomes.

The resting gene is subject to spontaneous mutation even in the absence of cellular metabolism or noxious chemicals and radiations (6, 7). Two heat-promoted processes predominate in bacteriophage T4. In one, 5-hydroxymethylcytidine residues deaminate to 5-hydroxymethyluridines, leading to G C \( \rightarrow \) A T transitions (2). The corresponding reaction in cellular DNAAs converts cytidines into uridines, which are efficiently repaired unless they occur at 5-methylcytidine residues, in which case thymidines are produced and are less efficiently repaired (5). In the other process, guanosine residues appear to be altered in a manner that generates transversions (3). The target base was identified as guanosine rather than cytidine because transversion mutations from G C base pairs were expressed immediately when G resides on the transcribed strand but only after DNA replication when G resided on the complementary strand (3).

Although genetic evidence identified heat-induced transversions at G C base pairs (for instance, by the reversion of amber mutations), it did not distinguish between G C \( \rightarrow \) C G and G C \( \rightarrow \) T A. However, the former pathway appeared reasonable because some heat-induced HII mutants are revertible by hydroxylation to a pseudo-wild state with a weakly mutant plaque phenotype (7), suggesting the pathway G C \( \rightarrow \) C G \( \rightarrow \) T A. Seeking a resolution of the two possibilities, we collected spontaneous and heat-induced revertants of two HII amber mutants and determined their DNA sequences. Only the G C \( \rightarrow \) T A pathway was appreciably induced.

MATERIALS AND METHODS

Media. L broth, Drake top and bottom agar, and our modified M9CA medium are described in reference 4 and references therein.

Strains. T4 stocks were grown on Escherichia coli BB, which selects neither for nor against HII mutants. HII* revertants were selected on strain KB, a lambda lysogen. The HII amber mutants rHII29 and rNT332 contain mutations at positions 195 and 540, respectively, in the numbering system of the HH870 HII HindIII DNA fragment (18). Bacteriophage stocks were grown in BB cells in L broth or M9CA medium, purified and concentrated by differential centrifugation (10), and suspended in 10 mM sodium phosphate buffer (pH 7.0) 0.5 mM MgCl2 at \( \approx 10^{33} \) PFU/ml.

Mutagenesis. Purified phage suspensions were diluted in 10 mM sodium phosphate buffer (pH 7.0) 0.5 mM MgCl2. A sample was removed from the diluted mixture and refrigerated at 4°C for 48 h for analysis of spontaneous revertants, while the remainder was incubated at 60°C for 48 h for analysis of heat-induced revertants. Samples were passaged through BB cells to resolve mutational heteroduplexes (3), the infected BB cells being prematurely lysed in order to restrict burst sizes to small values. The progeny phages were then plated on BB cells to determine total viable counts and on KB cells to determine HII revertant counts. The experimental conditions ensured that the sampled revertants were virtually all of independent origin.

DNA sequencing. Methods for genomic DNA preparation and sequencing with \( ^{32} \)P-end-labeled primers have been described previously (10); primer 1 was used for the rNT332 mutant and its revertants, and primers 5 and 6 were used for the rHII29 mutant and its revertants. Primers were purchased from Research Genetics (Huntsville, Ala.). Primer 1 includes base pairs 631 to 650 (Hammer) in the HII4 region. Primer 5 includes base pairs 224 to 258 (35-mer), and primer 6 includes base pairs 101 to 120 (20-mer) in the HII6 region.

RESULTS

The HII29 and rNT332 mutants carry heat-revertible amber (UAG) mutations whose DNA cognates are \( ^{32} \)P-end-labeled by missense mutations, so that spontaneous revertants with reversions at all three positions in a codon are likely to be recovered and both tyrosine codons are likely to be detectable. Extracistronic suppressors of HII amber mutations occur rarely, if at all.) G C \( \rightarrow \) A T transitions induced by 5-hydroxymethylcytidine deamination will convert the amber codon into an ochre codon and thus go undetected, whereas transversions will generate tyrosine codons and are likely to be detected.

We therefore determined the DNA sequences of 9 spontaneous and 10 heat-induced revertants of the rHII29 mutant and 9 spontaneous and 8 heat-induced revertants of the
rNT332 mutant (Table 1). Although spontaneous reversion occurred at diverse codon positions, all but one revertant of each heated amber mutant arose via a G · C → T · A transversion. The heat-induced increase in reversion frequency was ≥55-fold for the rHB129 mutant and 38-fold for the rNT332 mutant. The expected content of CAG revertants from the spontaneous background among the 10 rHB129* revertants from the heated sample was (10 × 7.7)/53 = 1.5, which accounts for the single observed CAG revertant. The expected content of TAT revertants from the spontaneous background among the eight rNT332* revertants from the heated sample was (8 × 0.8 × 6)/(23 × 9) = 0.2, indicating that all of the TAT revertants were probably heat-induced. The absence of CAG revertants from the nine spontaneous rNT332* revertants renders impossible any assessment of the single CAG revertant among the eight revertants from the heated sample.

In summary, the only transversion appreciably induced by heat in bacteriophage T4 is G · C → T · A.

DISCUSSION

Mutational specificity. The G · C base pair is the target for heat-induced transversions in bacteriophage T4, and guanosine appears to be the target base (3). Because the pathway is now seen to be G · C → T · A, the mispair is likely to be G* · A, where G* indicates the modified guanosine.

Mechanism. The formation of G* displays an activation energy of about 145 kJ/mol, a clear dependence on pH (the rate being nearly independent of pH over the interval 8 to 5 but increasing strongly over the interval 5 to 3.5), and a modest dependence on [Mg²⁺] (the rate increasing about 3.6-fold over the interval 0.05 to 10 mM) (3). These properties are consistent with a role for depurination in the transversion process. However, simple misincorporation opposite an apurinic template site is an inadequate explanation of the transversion process for three reasons. First, although adenosines depurate about 70% as rapidly as do guanosines (12), extensive tests failed to detect heat-induced base pair substitutions at A · T base pairs (3). Second, depurination mutagenesis in E. coli requires error-prone repair (21), but heat-induced transversion mutagenesis in T4 does not (3). Third, simple depurination mutagenesis involves the misinsertion of any base (11, 20), whereas we detected mispairing only with adenine. Therefore, a glycosyl bond migration reaction in which depurination is followed by repurination at a nitrogen other than N-9 was suggested previously (3); this is consistent with the ready condensation of 2-aminopurinylimidate and ribose-5-phosphate (14). Because heat-induced G · C → T · A transversions arise in clones that are small fractions of the total burst size (19), G* appears to miscode only infrequently and mostly to pair correctly with C.

Previously, only G* · purine mispairs that appeared to fit comfortably within the traditional dimensions of the B form of the DNA double helix were considered interesting mispairing candidates (3). Recently, however, spectroscopic analyses of purine · purine mispairs have revealed that even unmodified G · G* base pairs can be accommodated, albeit with reduced stability (9, 17). Furthermore, carbon-bonded protons have now become candidates for participation in hydrogen bonds (16, 22). We therefore searched for potential G* base pairs by using only loose constraints on glycosyl bond distance and angle and estimating the potential of guanosine depurinated at any nitrogen and in either its syn or anti configuration to pair with any base. Under these conditions, most G* configurations could be paired with all four normal bases. We found no grounds for predicting that a G* · A was preferred over a G* · G. There was no single depurinated guanosine family with sin-anti and/or N-1-N-3 proton localization that seemed likely to pair well with C, less well with A, and not with G or T, as might be expected from the clone size distribution experiments (19). Therefore, until more general and quantitative rules are developed to accurately predict mispairing potentials, we cannot distinguish among numerous and diverse depurination-repairation models of heat-induced transversion mutagenesis. Furthermore, because heat is a ubiquitous cause of DNA damage, DNA polymerases may have evolved the ability to sense G* and to direct the incorporation of cytosine opposite G* with little regard to the pairing configuration.

Of course, both common sense and clonal analysis (19) dictate that G* could also consist of some rare kind of heat-induced damage quite different from glycosyl bond migration. In addition, because we have examined events at only two DNA sites, other mispairs (such as G* · G) might be detected at other sites.

Implications for large, warm genomes. In bacteriophage T4 at 37°C, the heat-induced transversion mutation rate is about 4 × 10⁻⁹ per G · C base pair per h (3). For humans, with a generation time of about 20 years and a haploid genome containing about 1.4 × 10⁹ G · C base pairs, the corresponding rate will be about 10⁶ mutations per germ cell, provided the relevant chemistry is similar in T4 particles and in chromatin. Even if this number were to be reduced by physiological factors such as the ionic environment and the reduced scrotal temperature of the nude male and further reduced by the unknown fraction of DNA in which mutation is irrelevant, it would remain sufficiently large to constitute a genetic hazard. It is therefore likely that efficient repair.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Originᵃ</th>
<th>Spontaneous revertants</th>
<th>Heat-induced revertants</th>
<th>Fold increaseᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency (Sequence(s) (no. of revertants))</td>
<td>Frequency (Sequence(s) (no. of revertants))</td>
<td></td>
</tr>
<tr>
<td>rHB129</td>
<td>CAG → TAG</td>
<td>7.7 × 10⁻⁷ CAG (9)</td>
<td>53 × 10⁻⁷ CAG (1)</td>
<td>≥55</td>
</tr>
<tr>
<td>rNT332</td>
<td>TCG → TAG</td>
<td>0.8 × 10⁻⁷ TAT (6)</td>
<td>23 × 10⁻⁷ TAT (7)</td>
<td>38</td>
</tr>
</tbody>
</table>

ᵃ From reference 20.
ᵇ Calculated as [(revertant frequency in heated sample) × (TAT fraction)] ÷ [(revertant frequency in control sample) × (TAT fraction)].

TABLE 1. DNA sequences of rll amber mutants and their spontaneous and heat-induced revertants
processes operate in healthy individuals to reduce this hazard. Such a process might be akin to the E. coli mutY system, which specifically removes the A moiety of a G·A mispair (1, 13, 15).

Implications for small, hot genomes. Some bacteria grow at a temperature in the neighborhood of 100°C. For the recently described Methanopyrus spp. (8) growing optimally at 98°C with a doubling time of 50 min, ~400 heat-induced transversions will occur per generation. At 110°C, at which the doubling time is 8 h, the figure rises to ~3,000 per generation. The caveats for extrapolating from bacteriophage T4 also apply here. However, extreme thermophiles may possess additional protective measures, such as heat pumps.

LITERATURE CITED