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Mutagenesis: Interactions with a parallel universe☆

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ABSTRACT

Unexpected observations in mutagenesis research have led to a new perspective in this personal reflection based on years of studying mutagenesis. Many mutagens have been thought to operate via a single principal mechanism, with secondary effects usually resulting in only minor changes in the observed mutation frequencies and spectra. For example, we conceive of base analogs as resulting in direct mispairing as their main mechanism of mutagenesis. Recent studies now show that in fact even these simple mutagens can cause very large and unanticipated effects both in mutation frequencies and in the mutational spectra when used in certain pair-wise combinations. Here we characterize this leap in mutation frequencies as a transport to an alternate universe of mutagenesis.

1. Introduction

The concept of parallel universes (see Fig. 1) has intrigued science and science fiction writers, *e.g.* [1–3], as has the amusing "Hitchhiker's Guide to the Galaxy" description of someone's words falling through a "wormhole in the fabric of the space-time continuum" and being instantly transported to a distant galaxy in a completely different time frame [4].

When we reflect on what we currently understand about mutagenesis, we can in fact envision different modes of mutagenesis involving the same mutagenic agent as being in parallel universes. This can be seen with recent published revelations concerning base-analog mutagens [5], once thought to be the simplest of mutagens, yet seen in combination their actions are more complex. When I published my first paper on mutagenesis 40 years ago [6], I thought mutagens such as 2-aminopurine (2AP) acted only by straightforward mispairing, as originally conceived by pioneers such as Benzer, Freese, Brenner, Crick, and others [7–10], and as reproduced in reviews and standard textbooks from that point until the present day, *e.g.* [11–13]. Only now do I appreciate the importance of the alternate pathways by which these agents can act and even interact with one another.

2. Base analog mutagenesis in the standard universe

Base analogs are derivatives of the normal bases, adenine (A), guanine (G), cytosine (C), and thymine (T). Normally, when in a DNA double helix, these four bases pair according to the Watson-Crick pairing, A with T, and G with C. Rare tautomers can mispair, leading to incorrect bases being inserted during replication that will result in mutagenesis if not corrected. Fig. 2 shows the structures of a set of base and nucleoside analogs.

Compounds such as 2-aminopurine (2AP; recall that adenine is simply 6-aminopurine) can mispair with cytidine more frequently than adenine does, because the mispairing tautomers are more frequent. Often the actual tautomers are different than those originally envisioned (see Fig. 3) [14,15], but the concept is the same. Likewise, analogs of cytidine, such as zebularine (ZEB; cytidine lacking the amino group) [16] can make mispairs apparently more frequently than cytidine itself.

Each base or nucleoside analog generates mutations by its specific mispairing. We can ascertain this by comparing the mutagenic spectra of each mutagen at an array of sites. The *Escherichia coli rpoB* gene is an excellent target for this, as it has as many as 92 base-substitution mutation possibilities detected so far [17–19] that lead to the rifampicin resistant phenotype (Rif^r). There are 28 transitions among these possible mutations (*i.e.* A:T ≥ G:C or G:C ≥ A:T). Seymour Benzer's elegant studies of the *r*II system in phage T4 first demonstrated that different mutagens have different favored sites (hotspots) [8], and we can see that reflected here in the *rpoB* gene for several mutagens or mutators (Fig. 4). This shows that rather than each activating the same process, instead, they each have an individual "fingerprint." There are indirect mechanisms of mutagenesis that base analogs could in principle activate (see below), but they do not seem to do this when these compounds act alone to any degree that changes the fingerprints seen in

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Fig. 1. A representation of the different parallel "worlds" that might exist in other pockets of the multiverse. Image credit: public domain, retrieved from https://pixabay.com/en/globe-earth-country-continents-73397/; see [1].

Fig. 4. These other mechanisms of mutagenesis are part of a different universe.

3. The universe of dNTP ratios

There is another form of mutagenesis, totally apart from that caused by direct acting mutagens, that emanates from changing the deoxyribonucleoside triphosphate (dNTP) ratios, the building blocks of DNA that fuel replication. Microbial and higher cells maintain a precise balance of dNTP levels and ratios, and replication speed *e.g.* [20,21]. Changes in dNTP pools affect mutation rates [22–33]. Relatively small changes in the ratios of the 4 different dNTPs can result in large increases in replication errors and subsequent mutation rates, *e.g.* [23,24]. We know this because of the analysis of different mutants with altered dNTP ratios. Mutants defective in DCD (deoxycytidine deaminase) or NDK (nucleotide diphosphate kinase) have increased dCTP and dGTP [21,23,25,26], and decreased dATP [23,25] and higher rates of certain base substitutions [23,25–27,34]. The double mutant deficient in both DCD and NDK has a larger imbalance and a more extreme mutation rate increase [24]. Moreover, mutants carrying engineered alterations of RNR (ribonucleotide reductase), which controls the ratios of dNTPs through a set of allosteric sites, have high mutation rates and specific alterations in dNTP levels [35] (see below). Changes in the absolute levels of dNTP also affect mutation rates. Thus, when the levels of all dNTPs are increased, mutation rates increase [31,32], and when they are all decreased, mutation rates are lowered [33].

Base analogs may alter dNTP ratios, and this was appreciated already during earlier studies of mutagens such as 2AP and 5-bromodeoxyuridine (5BrdU) [36–38]. Interestingly, increasing the concentration of single base analogs in mutagenesis studies does not change the ratios past the tipping point needed to stimulate this latter type of mutagenesis. Thus, both of these modes of mutagenesis are stuck in their own universe.

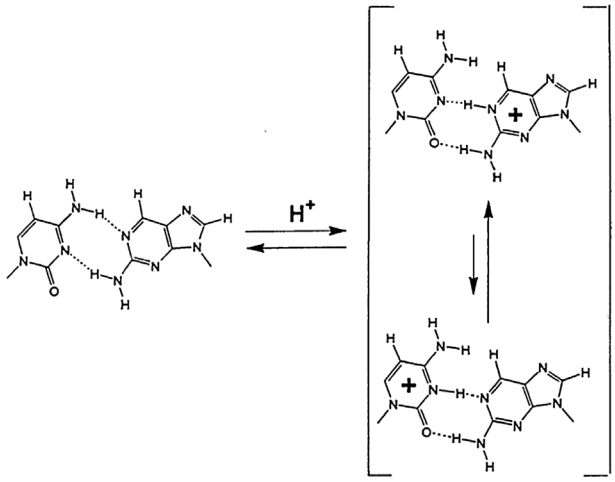

Fig. 3. Structures of the possible AP-C mispair hydrogen-bonded configurations. Left, neutral wobble; right-upper, ionized Watson-Crick configuration, protonated on the AP residue; right-lower, ionized Watson-Crick configuration, protonated on the C residue. Figure reproduced from [14] with permission of the publisher (American Chemical Society).

4. Transition from one universe to another

We recently began a study of the effects of combinations of mutagens, beginning with base analogs [5]. Although there have been studies of compounds that act as mutagen enhancers by inhibiting specific repair enzymes [39,40] or inhibiting enzymes that inactivate certain mutagenic compounds [41–44], and also studies showing that intercalating agents can increase the mutagenicity of bleomycin by perhaps increasing their access to the DNA [45,46], the topic of mutagen synergy still remains a vastly unexplored field. We were surprised to find that certain pairwise combinations of base analogs gave strong synergistic or even antagonistic or suppressive effects. The combination of ZEB and 2AP gave the largest effect, increasing mutation frequencies 35-fold over that from the addition of frequencies from both mutagens used alone (Fig. 5).

The combination of 2AP and 5-azacytidine (5AZ) generated a much smaller increase. Also, the combination of 2AP and 5-bromodeoxyuridine (5BrdU) actually gave a 7-fold decrease in the frequency expected from adding the two individual frequencies (Fig. 6).

What can cause the dramatic synergistic effect in mutation frequencies seen in the 2AP + ZEB pairwise combination? We can examine the "fingerprint" revealed by looking at the mutational spectrum in the *rpoB* gene and try to compare it with that from other mutagens or

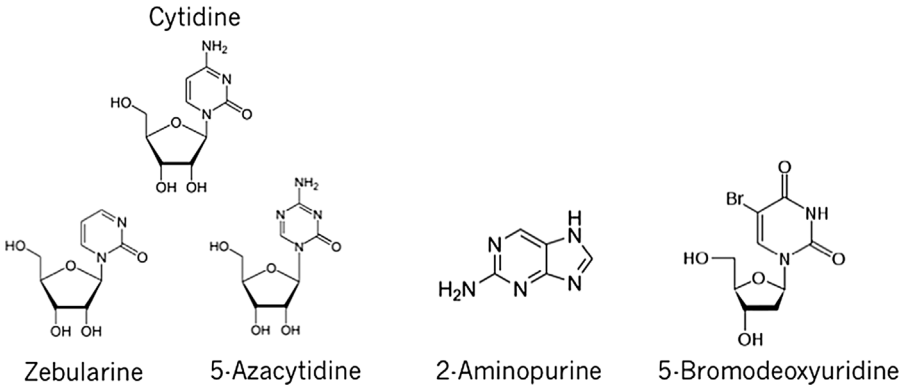

Fig. 2. The structure of four base or nucleoside analogs discussed here (Figure adapted from [5]).