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Deoxyribonucleotide pools, base pairing, and sequence configuration affecting bromodeoxyuridine- and 2-aminopurine-induced mutagenesis

(bromouracil substitution for cytosine/deoxyribonucleoside triphosphate synthesis/DNA fidelity/mutagenic hot spots)

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ABSTRACT **Despite recent experiments showing that BrdUrd-induced mutagenesis can be independent of the level of bromouracil (BrUra) substitution [Kaufman, E. R. & Davidson, R. L. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4982–4986; Aebersold, P. M. (1976) *Mutat. Res.* 36, 357–362], BrUra·G base mispairs are a major determinant of mutagenesis. We propose that the experiments cited above are sensitive predominantly to G·C→A·T transitions driven by the immeasurably small but highly mutagenic substitution of BrUra for cytosine and not by the gross substitution of BrUra for thymine in DNA. More generally, we show how accumulated evidence suggests that both BrdUrd and 2-aminopurine have two mutagenic effects intracellularly: perturbation of normal deoxyribonucleoside triphosphate pools, and analogue mispairs in DNA. We propose a molecular basis for various observations of normal exogenous deoxyribonucleosides as synergists and counteragents to base analogue mutagenesis. A model is proposed to explain the antipolarity of BrdUrd and 2-aminopurine mutagenesis—i.e., why mutants at hot spots for induction by one base analogue are usually hot spots for reversion by the other. It is concluded that the configuration of the neighboring nucleotides surrounding the base analogue mispair, and not the base analogue's preference for inducing A·T→G·C or G·C→A·T errors, is responsible for the antipolarity of BrdUrd and 2-aminopurine mutagenesis.**

5-Bromouracil (BrUra), an analogue of thymine, and 2-aminopurine (APur), an analogue of adenine, cause transition mutations in various organisms (1–14). A·T→G·C and G·C→A·T transitions are induced by both base analogues; however, the extent of their bidirectionality remains unclear (8–21). It is known that BrUra and APur are incorporated "normally" into DNA opposite adenine and thymine, respectively. Ambiguities in their base pairing properties (BrUra can pair with guanine and APur with cytosine) are clearly important determinants in their mutagenicity and lead to what Freese (9) and Rudner (19) defined as replication errors and incorporation errors.

Under certain conditions, BrUra substitution in DNA is not correlated with BrdUrd mutagenesis. This point was forcefully made in recent experiments by Kaufman and Davidson (22) using Syrian hamster melanoma cells and by Aebersold using Chinese hamster cells (1). For example, in the Kaufman and Davidson experiment it was shown that BrdUrd-induced mutation frequencies in a Syrian hamster melanoma cell line depended not on how much BrUra was stably substituted for thymine in the DNA but rather on the concentration of BrdUrd in the cell growth medium. In view of the evidence in *Salmonella* (14) and in bacteriophage T4 (23), which supports the idea that BrUra increases replication errors when acting in a template capacity, the Syrian hamster melanoma results are remarkable, to say the least.

It will be of fundamental importance in understanding the mechanism of BrdUrd mutagenesis to recognize that the uncoupling of the level of BrUra substitution in DNA from the level of BrdUrd mutagenesis was achieved through the addition of deoxythymidine or deoxycytidine to the BrdUrd mutagenesis medium (1, 2, 22). The addition of deoxythymidine resulted in less, or even undetectable, BrUra substitution in DNA but the same, or increased, level of mutagenesis (1, 2, 22). Conversely, the addition of deoxycytidine with BrdUrd during mutagenesis resulted in a complete inhibition of mutagenesis but full substitution of BrUra for thymine in DNA (2).

These observations have led some investigators to conclude that BrdUrd mutagenesis occurs because of an altered physiological state such as "deoxycytidinelessness" (24) or to implicate BrdUrd metabolites as allosteric effectors of the fidelity of DNA synthetic enzymes (1). It has also been proposed that BrdUrd mutagenesis does not involve mispairing of BrUra during DNA synthesis (5, 10, 22, 25).

The conclusion that BrUra base pairs exactly as thymine conflicts with certain experiments termed "clean growth" (23) in which BrUra-substituted DNA was proved to induce replication errors in the absence of exogenous BrdUrd at the same rate that mutagenesis occurs during standard BrdUrd treatment. Because this is a site-specific phenomenon, occurring at an enormous rate at some genetic loci and not at all at other BrdUrd-mutable loci (23), this phenomenon is not due to the breakdown of BrUra-substituted DNA resulting in normal BrdUrd mutagenesis. The clean growth experiments show that BrUra substitution at certain loci drive replication errors, presumably through BrUra·G mispairs, in apparent contradiction to the interpretations of Aebersold (1) and Kaufman and Davidson (2, 22, 26).

In this communication we explain how apparently conflicting observations of BrdUrd mutagenesis in various prokaryotes (3–11) and eukaryotes (1, 2, 22) can be reconciled. The effects of BrdUrd mutagenesis are 2-fold, involving perturbation of normal deoxyribonucleoside triphosphate pool sizes (24) *and* base-pairing ambiguity. We shall emphasize the importance of considering the specific nature of the genetic transition (i.e., A·T→G·C or G·C→A·T) that one assays when interpreting mutagenesis data. Each of the two effects of BrdUrd treatment is expected to preferentially induce one type of transition, and we analyze the ability of normal deoxyribonucleosides to act both as synergists and counteragents when added during BrdUrd mutagenesis.

We will also explain how the inhibition of adenosine deaminase (9, 27) by APur-deoxyribose leads us to predict that the mutagenic effects of APur are also 2-fold: perturbation of normal deoxyribonucleoside triphosphate pool sizes and the well-documented base-pairing ambiguities (9, 12, 28–33).

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Abbreviations: BrUra, 5-bromouracil; APur, 2-aminopurine; ADAase, adenosine deaminase.

A second topic regarding APur and BrdUrd mutagenesis will be discussed: their antipolarity. There seems to be conflicting evidence concerning whether BrUra and APur preferentially induce A·T→G·C transitions more frequently than G·C→A·T transitions or vice versa (8–21). Indeed, each analogue seems to be able to induce both transitions, because BrdUrd-induced mutants can be induced to revert by BrdUrd to some extent and APur-induced mutants can be induced to revert by APur (34). However, Champe and Benzer's (15, 35) extensive characterization of BrdUrd- and APur-induced mutants shows that nearly all the BrdUrd-induced mutants arose as G·C→A·T transitions, but that the APur-induced mutants arose half from G·C→A·T and half from A·T→G·C transitions. The issue is clouded by Freese's finding (34) that mutants at "hot spots" for BrdUrd induction are extremely revertable by APur and, conversely, that mutants at hot spots for APur induction are extremely revertable by BrdUrd. He concluded from this antipolarity that APur preferentially induces A·T→G·C transitions and BrdUrd induces G·C→A·T transitions.

We present a model of BrdUrd and APur mutagenesis at hot spots which predicts that the antipolarity of BrdUrd and APur mutagenesis is due to the configuration of the neighboring sequence at the site on DNA where mispairing occurs rather than to any preference of the analogue to induce A·T→G·C or G·C→A·T transitions.

Mechanism of BrdUrd mutagenesis

BrdUrd and its nucleotides compete with thymidine and its nucleotides as substrates in the salvage pathway leading to deoxyribonucleoside triphosphates. Depending on the amount of deoxythymidine and BrdUrd present in the medium and upon the amount of residual *de novo* pathway activity, BrdUTP concentrations *in situ* can exceed TTP concentrations. This is evidenced by the ease with which BrUra can substitute almost completely for thymine in DNA (1, 7, 18, 22, 36–38). Let us first consider the effect of excess BrdUTP on the A·T→G·C transition. Once BrUra has replaced thymine, a BrUra·G base pair can be formed when the BrUra-containing template is replicated. As suggested by Freese (9), comparison of equilibria between *keto* and *enol* forms for the bases BrUra and thymine leads to the conclusion that BrUra·G mispairs are more likely to form than T·G mispairs; on this basis it was logical for Freese to propose that BrUra·G replication errors cause A·T→G·C transitions at the original BrUra incorporation sites. Ionization of BrUra may also enhance the ambiguity in its base-pairing properties (39).

But subsequent experiments have also demonstrated that replacement of a large fraction of thymine in the DNA (even 100%) is not necessarily mutagenic (1, 22, 26, 36, 37). Does this observation imply that the mutagenic effects of BrdUrd cannot depend upon the amount of BrUra in DNA or even require that BrUra be incorporated into DNA? We will argue that the answer is "no"—that is, BrdUrd-induced A·T→G·C transitions still occur directly as a result of BrUra substitution for thymine in DNA. To reconcile the previous statement with the failure in some instances to observe a correlation between BrUra substitution in DNA and mutagenesis, we must first consider a second mode of action for BrUra which relates only indirectly to base pairing and which selectively drives the BrdUrd-induced G·C→A·T transition.

BrdUTP is an inhibitor of ribonucleoside diphosphate reductase. This inhibition mainly affects the reduction of cytidine diphosphate to deoxycytidine diphosphate, and high levels of dTTP, dGTP, and dATP are generally synergistic with BrdUTP in this inhibition (24, 40, 41) (see review of ribonucleotide reductase in ref. 42). The mutagenic implications are simple to understand. High BrdUTP concentrations *in vivo* will prevent the formation of the cytosine substrate for DNA synthesis, dCTP. With a decrease in dCTP pools, BrdUTP will become increasingly competitive for sites opposite template guanines during DNA replication. Therefore, as a result of the ambiguous base-pairing properties of BrUra and because it is likely that BrdUTP pools will be much larger than either dCTP or TTP pools, the formation of BrUra·G base pairs will be enhanced significantly during DNA replication. This is tantamount to stimulating G·C→A·T transitions because, in contrast with A·T→G·C transitions discussed earlier, a BrUra·G base pair is now formed with G on the template and BrdUTP as an incoming substrate. Freese (9) and Rudner (19) named this mispairing configuration a "misincorporation error." The important point is that BrUra-induced G·C→A·T transitions are driven *both* by base mispairing frequencies and by dCTP pool size reductions, whereas dCTP pool size should not influence BrUra-induced A·T→G·C transitions.

The studies which uncoupled BrUra substitution and mutagenesis (1, 2, 22) used assays of mutagenesis that may be sensitive only to G·C→A·T transitions. In several studies, the addition of thymidine during BrdUrd mutagenesis resulted in a lower level of BrUra substitution whereas the rate of mutagenesis remained fairly constant (1, 22, 26, 36). Although it may therefore be tempting to conclude that BrUra need not be incorporated into DNA to exert a mutagenic effect, we contend that these observations neither prove nor even imply that BrdUrd mutagenesis does not require mispairing BrUra with guanine.

To explain, it has been shown that the addition of thymine or deoxythymidine to the growth medium causes an increase in TTP pool concentrations (43, 44). It follows that BrUra substitution into DNA will be decreased because BrdUTP will be a less effective competitor against TTP for sites opposite template adenines. This would also decrease BrdUrd-induced A·T→G·C transitions but could increase BrdUrd-induced G·C→A·T transitions. This would happen because an increased pool of TTP can also inhibit CDP reductase activity, decreasing even further the concentration of dCTP in the cell (41, 45). Thus, BrdUTP becomes a better competitor against dCTP for sites opposite template guanines, but it becomes less competitive against TTP for sites opposite template adenines. Similar schemes of mutagenic mispairs driven by perturbed deoxyribonucleoside triphosphate pools have been proposed (26, 45–48).

We conclude that, although the net substitution of BrUra for thymine in DNA decreases, the highly mutagenic but immeasurably small substitution of BrUra for cytosine increases. It follows logically that the mutagenesis measured by Kaufman and Davidson (22, 26) and by Aebersold (1) could be occurring at specific hot spots for BrdUrd-induced G·C→A·T transitions. Thus, despite the fact that the mutation frequency that they assayed is not proportional to the total amount of BrUra substituted in the DNA, a primary determinant of mutagenesis may still be the misincorporation of BrUra into DNA opposite a template guanine.

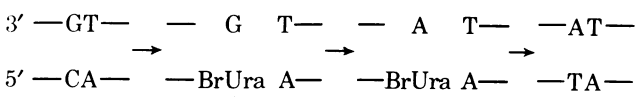
The mutagenicity, toxicity, and even the effects on differentiation associated with BrdUrd treatment can often be counteracted by the addition of deoxycytidine (2, 24, 26, 37). It has been proposed that the "physiological state" of deoxycytidinelessness (24) rather than the incorporation of BrUra into DNA is responsible for BrdUrd mutagenesis and that therein lies the explanation of deoxycytidine as a counteragent. An alternative way to understand deoxycytidine as a counteragent to BrdUrd mutagenesis involves BrUra incorporation, BrdUrd perturbation of deoxyribonucleoside triphosphate pools, and

the nature of the site(s) undergoing transition. BrdUrd leads to a deficiency in dCTP by a block in the *de novo* deoxyribonucleotide synthetic pathway as explained earlier (24, 42, 49). This block can be overcome by the addition of deoxycytidine because the salvage pathway kinases (which are independent of ribonucleotide reductase) can convert the deoxyribonucleoside to a deoxyribonucleoside triphosphate. When dCTP is present, BrdUTP will not compete effectively for sites opposite guanine on DNA. Thus, BrdUrd will not induce G·C→A·T transitions [which are the predominant mode of BrdUrd mutagenesis, (15)] when deoxycytidine is provided exogenously, but BrUra may still replace thymine in DNA to a great extent. The effectiveness of deoxycytidine as a counteragent to BrdUrd mutagenesis adds support to our contention that the Kaufman and Davidson measurement (2, 22, 26) is selectively sensitive to G·C→A·T transitions.

Thymidine can also behave as a counteragent to BrdUrd mutagenesis (1, 11, 50). Obviously, A·T→G·C transitions will be counteracted when exogenous thymidine is converted to TTP, which competes with BrdUTP for sites opposite template adenines. Thus TTP prevents BrUra substitution for thymine in DNA, the first step in the BrdUrd-induced A·T→G·C transition pathway. By a second mechanism, small amounts of thymidine might also counteract BrdUrd-induction of G·C→A·T transitions. Here, thymidine would compete with BrdUrd as a substrate for thymidine kinase. This would prevent the conversion of BrdUrd to BrdUTP, which in turn would prevent the BrdUTP inhibition of CDP reductase activity. Thus, as long as TTP does not become concentrated enough to inhibit CDP reduction itself, the addition of deoxythymidine at a low concentration may inhibit BrdUrd-induced G·C→A·T transitions. The extent to which TTP pools are increased by the addition of exogenous thymine or deoxythymidine will vary as the degree of TTP feedback inhibition of thymidine kinase varies from one organism to another. For example, TTP pools are much more strongly correlated with the concentration of exogenous thymine in T4-infected *Escherichia coli* than in uninfected *E. coli* (43).

Purine deoxyribonucleosides, as described earlier for thymidine, can also act as synergists to BrdUrd mutagenesis (11, 26) by inhibiting CDP reductase and thus enhancing G·C→A·T transitions. However, another effect on mispairing of a more subtle nature results when there is an increase in the pool of any deoxyribonucleoside triphosphate. The concentration of deoxyribonucleoside triphosphates has been shown to affect the kinetics of incorporation of incorrect nucleotides (29). In particular, it has been shown that misincorporation frequencies *in vitro* increase with increasing substrate concentration because a 3′-exonuclease error-correcting activity (51, 52) [known to be associated with prokaryotic DNA polymerases (53)] is less likely to remove a mispaired terminal nucleotide whenever the proper dNTP substrate is available for chain elongation (29, 54).

Consider, for example, the synergism of deoxyadenosine with BrdUrd mutagenesis, in which a G·C→A·T transition occurs at a site next to a T·A base pair:



We can expect the addition of deoxyadenosine to drive this transition if the following three conditions are met: (*i*) the addition of deoxyadenosine results in an increase of dATP pools *in situ*, (*ii*) error correction of the BrUra·G base pair takes place prior to the incorporation of dATP on the 3′ side of the BrUra, and (*iii*) increase of the dATP pool increases the rate of dATP incorporation (e.g., see refs. 29 and 54). If these conditions are met, then an increase in dATP pools will cause the BrUra·G base pair to be rapidly "sealed" in place, protected from the action of a 3′-exonuclease editing activity. For this example, deoxyadenosine acts synergistically with BrdUrd to drive the G·C→A·T transition. By similar arguments one can conclude that increasing the rate of formation of the normal base pair on the 3′ side of any mismatched base pair will increase the probability of a mispair surviving to be scored as a mutation.

These effects of exogenous deoxyribonucleosides should be site-specific. Deoxyadenosine will be synergistic for sites where adenine is the 3′ adjacent base of the mispair, deoxyguanosine will be synergistic for sites where guanine is the 3′ adjacent base of the mispair, and so forth. However, synergism of this nature is independent of the direction of the transition. Depending again on the 3′ neighbor of the site in transition, any deoxyribonucleoside might be synergistic for A·T→G·C transitions or for G·C→A·T transitions. We would expect this effect to be much smaller in magnitude than deoxyribonucleoside triphosphate pool size effects.

Pool size effects on APur mutagenesis

As an analogue of adenine, APur can base pair with thymine (9); as an analogue of guanine, it can also pair with cytosine (9, 32, 33). By using T4 bacteriophage that codes for mutator, antimutator, or wild-type DNA polymerase, it has been observed that the incorporation of APur into DNA correlates with APur mutagenesis (30). As in the case of BrdUrd, we believe it very likely that both analogue base mispairing and nucleotide metabolism must ultimately share a central role in APur mutagenesis. However, the effects of APur on nucleotide metabolism are not well documented. Nevertheless, strong circumstantial evidence leads us to speculate that the effects of APur metabolites leading to mutagenesis are 2-fold.

First, indirect (9) and direct (27) evidence shows that the APur metabolite APur-deoxyribose is an extremely potent inhibitor of adenosine deaminase (ADAase). ADAase is a catabolic enzyme usually considered to be part of the salvage pathway for purine nucleotide and deoxyribonucleotide synthesis. It catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. Normally, this deamination process is rapid enough [K_m for ADAase = 7 μM (55)] to prevent the phosphorylation of deoxyadenosine [K_m for deoxyadenosine kinase = 400 μM (56)]. However, in ADAase$^-$ cells, deoxyadenosine is rapidly phosphorylated, resulting in toxic concentrations of dATP (56–58). High intracellular pools of dATP strongly inhibit ribonucleotide reductase, thus dramatically decreasing the concentration of dGTP, TTP, and dCTP *in situ* (40, 58).

This line of reasoning leads us to predict that, during APur mutagenesis, 2-aminopurinedeoxyribose inhibition of ADAase may result in very high levels of dATP, which in turn would perturb the normal DNA precursor pools of dGTP, TTP, and dCTP. This perturbation of normal DNA precursors is expected to enhance mispairing probabilities during DNA synthesis, when the "wrong" precursor is more concentrated than the "right" precursor at a given site.

A second effect of APur is that its metabolite dAPTP will drive mutagenesis because the well-documented ambiguity in its base-pairing properties (12) facilitates mispairing during DNA replication, especially when the concentration of normal DNA precursors are limiting. Furthermore, APur apparently mispairs with cytosine much more frequently when APur lies on the template than when it serves as a deoxyribonucleoside triphosphate substrate (30–33). Thymineless mutagenesis is synergistic with APur mutagenesis in bacteriophage T4 (59).

This is true when the thymineless state is induced by 5-fluorodeoxyuridine, an inhibitor of thymidylate synthetase, or when the thymineless state is brought about through genetic blocks in deoxyribonucleotide synthetic pathways. We will expand a logical explanation for the molecular mechanism of this synergism (59). APur is incorporated into DNA opposite template thymine. During replication in the thymineless state, when the APur-containing strand serves as a template, dCTP becomes a significant competitor for a site opposite that template APur for two reasons. Low TTP concentrations *in situ* will cause more mispairing of dCTP with template APur. In addition, low levels of TTP *in situ* increase the pool of dCTP dramatically (60, 61), further driving the C·APur mispair. This will drive APur-induced A·T→G·C transitions but not G·C→A·T transitions. From the previous discussion it should be clear that, as in the case of BrUra, the effect of APur and its metabolites on DNA precursor synthetic pathways promises to be a crucially important factor in the elucidation of its mutagenicity.

Antipolarity of BrUra and APur mutagenesis at hot spots

APur and BrdUrd both induce A·T→G·C and G·C→A·T transition mutations. The A·T→G·C mutations arise from replication errors, and the G·C→A·T mutations arise from incorporation errors (9, 19). A mutant induced by BrdUrd can be induced to revert by BrdUrd; similarly, a mutant induced by APur can be induced to revert by APur (34). There is still considerable question as to whether one or both of these base analogues preferentially induces a transition in one direction more than in the opposite direction (e.g., A·T→G·C more than G·C→A·T) (8–21).

Benzer and Freese (8) isolated a large number of phage T4r$_{II}$ mutants at hot spots for spontaneous, BrdUrd-, and APur-induced mutagenesis. Freese (9) found that the spectrum of base analogue-induced mutations which are mostly transitions does not correlate with the spectrum of spontaneously induced mutations which are mostly transversions or frameshift mutations. Moreover, the spectrum of BrdUrd-induced hot spots included some sites that also were hot spots for APur induction. Freese also found that some sites were hot with respect to only one base analogue. Table 1 in Freese's manuscript (34) shows spontaneous and base analogue-induced reversion frequencies of mutants that were selected as hot spots for BrdUrd or for APur induction. Mutants at hot spots for BrdUrd induction are extremely revertable by APur. Likewise, mutants at hot spots for APur induction are extremely BrdUrd revertable. Freese surmised from the antipolar fashion in which one analogue efficiently reverts mutants induced by the other that there is a specificity inherent in APur mutagenesis that is distinct from the specificity inherent in BrdUrd mutagenesis. He surmised that BrdUrd induces G·C→A·T transitions preferentially (although not exclusively) whereas APur induces A·T→G·C transitions preferentially.

We now propose a model that attributes the specificity in the antipolarity of APur and BrdUrd mutagenesis observed by Freese (34) to the site on DNA undergoing transition, not to the base analogue that induces the transition. The model assumes that hot spots are determined by neighboring nucleotide sequences and is independent of the molecular mechanism of base analogue-induced mutagenesis, except that replication and incorporation errors require the analogue's incorporation into DNA. Effects due to base stacking and postreplicative repair have not been considered.

Because Freese was selecting hot spots for BrdUrd- and APur-induced mutagenesis, it is not necessary to assume that these base analogues preferentially induce transitions in one direction. We now have much direct and indirect evidence that neighboring nucleotides play a role in determining mutation frequencies for a given locus (13, 17, 18, 20, 35, 62–65). Because DNA is synthesized 5′ to 3′ and because either strand can serve as a template, two different sets of neighbors can be present during a mutagenic event at a single locus. The set of neighbors that are present is determined by the strand upon which the error occurs. Fig. 1 symbolizes a single locus and its neighboring nucleotides. It is apparent that the neighbors present during the incorporation error induced by BrdUrd (Fig. 1*c*) in the G·C→A·T direction are the same as the neighbors involved in the APur-induced replication error (Fig. 1*j*) in the A·T→G·C transition direction. The neighbors present at the moment the BrdUrd-induced replication error occurs (Fig. 1*b*) are different from the neighbors present when the same base pair undergoes the reverse transition stimulated by the same mutagen, BrdUrd (Fig. 1*c*).

In a similar fashion, APur-induced incorporation errors at a given locus will occur with different neighbors from APur-induced replication errors again at the same site. The BrdUrd-induced replication error occurs with the same neighbors as the APur-incorporation error. Conversely, the BrUra incorporation error has the same neighbors as the APur replication error. Thus, if mutational hot spots are determined by neighboring nucleotide sequences, then, *by definition*, a hot spot for BrdUrd induction is a hot spot for APur reversion and,

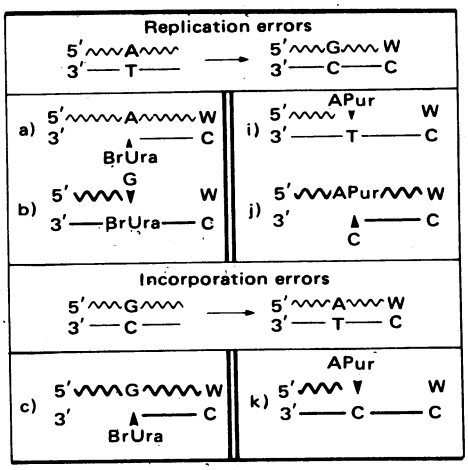


FIG. 1. Various configurations of a single DNA site and its relevant neighbors as the site undergoes A·T→G·C and G·C→A·T transition mutations induced by APur (*Right*) or BrUra (*Left*). One strand of the duplex is shown as a wavy line labeled "W"; this strand always carries a purine at the site shown in this example. The other strand of the duplex is shown as a straight line labeled "C"; this strand always carries a pyrimidine at the site in question. The important feature of this figure is the neighboring pieces of DNA present at the moment the replication or incorporation error is made. The G·BrUra base pair shown in *b* is the limiting step in the BrUra-induced A·T→G·C transition pathway. The neighbors involved are the whole C strand and the 5′ side of the W strand. These are the same neighbors involved in the limiting step, when APur induces the opposite G·C→A·T transition as APur mispairs with a template C shown in *k*. The G·BrUra base pair shown in *c*, which is the limiting step in the BrUra-induced G·C→A·T transition, is flanked by the whole W strand and the 5′ side of the C strand. Again, these are the neighbors involved in the opposite transition induced by APur. BrUra is readily incorporated into DNA. APur is strongly excluded. Therefore, the APur-induced A·T→G·C transition pathway really has two limiting steps, *i* and *j*, whereas the APur-induced G·C→A·T and both BrUra-induced transitions have just one limiting step. Note that the two limiting steps in the APur-induced A·T→G·C transition pathway occur on opposite strands, with different sets of neighbors.

conversely, a hot spot for APur induction is by definition a hot spot for BrdUrd reversion. It has been shown that the neighboring bases on the 5′ side and on the 3′ side of an error significantly affect error rates (13, 14, 17, 20, 54, 62–65). However, we do not yet know the relative importance of the 3′ compared to the 5′ neighbors. We have tacitly assumed in this model that an extended sequence on the 3′ side of the error but only the adjacent base on the 5′ side of the error play major roles in neighbor effects.

There is one subtlety to this scheme to which we wish to call attention. The APur-induced A·T→G·C transition actually consists of two limiting steps; first, the incorporation of APur replacing adenine, which itself is a rare event, and second, the misincorporation of cytosine opposite that template APur (30, 31). The other three transitions shown in Fig. 1 have just one limiting step. BrUra readily substitutes for thymine in DNA. This in itself is not always mutagenic (22, 26, 36). Therefore, there need not be any hot spot for BrUra incorporation in place of thymine as in Fig. 1*a*. APur, however, is effectively excluded from DNA (30, 31, 66, 67). So there will be hot spots for incorporating APur in place of adenine (Fig. 1*i*). For the APur-induced A·T→G·C transition, a given site has two configurations in which it might be hot, corresponding to a hot spot for incorporating APur in place of adenine and the replication error of incorporating dCTP opposite the template APur shown in Fig. 1*j*. According to our model of the antipolarity of APur and BrUra mutagenesis, we would expect a "double hot spot" of this sort to be highly inducible by APur, highly revertable by APur, highly inducible by BrdUrd, and highly revertable by BrdUrd. T4r$_{II}$ N101 (see ref. 34) may represent this type of site.

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