MOLECULAR AND CELLULAR BIOLOGY, Nov. 1984, p. 2449–2454
0270-7306/84/112449-06$02.00/0

Vol. 4, No. 11

Replication of DNA Containing 5-Bromouracil Can Be Mutagenic in Syrian Hamster Cells

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Received 14 May 1984/Accepted 8 August 1984

A new protocol for inducing mutations in mammalian cells in culture by exposure to the thymidine analog 5-bromodeoxyuridine (BrdUrd) was established. This protocol, called "DNA-dependent" mutagenesis, involved the incorporation of BrdUrd into DNA under nonmutagenic conditions and the subsequent replication of the 5-bromouracil (BrUra)-containing DNA under mutagenic conditions but with no BrdUrd present in the culture medium. The mutagenic conditions were induced by allowing BrUra-containing DNA to replicate in the presence of high concentrations of thymidine. This generated high intracellular levels of dTTP and dGTP, causing nucleotide pool imbalance. The mutagenesis induced by this protocol was found to correlate with the level of BrUra substituted for thymine in DNA.

The thymidine (dThd) analog 5-bromodeoxyuridine (BrdUrd) is an effective mutagen in procaryotic (3, 9, 16, 20, 23) and eucaryotic (1, 12, 13, 22) systems. A model for the mutagenic action of this base analog was based on the Watson and Crick scheme of base pairing and the suggestion that rare tautomeric shifts of the bases could change their pairing properties during DNA replication and thereby lead to mutations (9). This model suggests that due to its chemical properties, 5-bromouracil (BrUra) would undergo a tautomeric shift to the rare enol state, allowing it to mispair with guanine (Gua) more frequently than would thymine (Thy). Thus, two possible mechanisms of BrUra mutagenesis were proposed, errors of incorporation and errors of replication. Errors of incorporation were thought to occur when bromodeoxyuridine triphosphate (BrdUTP) mispairs with a Gua residue in replicating DNA, resulting in GC-to-AT transitions after subsequent rounds of replication. Errors of replication were thought to occur when a BrUra residue in replicating DNA mispairs with dGTP, resulting in AT-to-GC transitions.

Recent studies in mammalian systems suggested that BrdUrd mutagenesis was determined primarily by the concentration of BrdUrd to which the cells were exposed and was independent of the amount of BrUra incorporated into DNA in place of Thy residues (13). This and other studies (2, 7, 8, 14) provided evidence that BrdUrd mutagenesis in mammalian cells was driven by an increase in the intracellular ratio of BrdUTP to dCTP and occurred only during incorporation of BrdUTP into DNA and that mutations due to replication of BrUra residues in DNA did not occur. (We refer to this mutagenesis as "pool-dependent" because it is dependent upon the BrdUTP pool levels and not the level of BrUra incorporated into DNA.)

The experiments presented in this report describe a new protocol for BrdUrd mutagenesis in mammalian cells, allowing the unambiguous detection of mutations caused by the replication of BrUra residues in DNA. (We refer to this mutagenesis as "DNA-dependent" because it was dependent upon the level of BrUra incorporated into DNA and occurred when there was no BrdUTP in the cell.) The DNA-dependent protocol involved the incorporation of BrUra into DNA under nonmutagenic conditions, followed by the removal of BrdUrd from the culture medium and BrdUTP from the intracellular nucleotide BrdUTP pools. Mutations were then induced by allowing the BrUra-containing DNA to replicate under conditions of nucleotide pool imbalance.

MATERIALS AND METHODS

Cells and media. The cells used in this study were derived from the Syrian hamster melanoma cell line RPMI 3460 (18). These cells, called 2E, were selected for their ability to grow with high levels of BrUra in their DNA (4). The basic culture medium for all the cells was Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum (DMEM). Pool-dependent mutagenesis experiments utilized FBT medium, which is DMEM supplemented with 10 μM 5-fluorodeoxyuridine and the appropriate concentrations of BrdUrd and dThd. DNA-dependent mutagenesis experiments utilized FBTdC medium, which is DMEM supplemented with 10 μM 5-fluorodeoxyuridine–10 μM deoxycytidine (dCyd) and the appropriate concentrations of BrdUrd and dThd. For the selection of drug-resistant mutants, DMEM was supplemented with either 36 μM 2-thioguanine (Sgu) or 1 mM ouabain (Oua).

Protocol for pool-dependent mutagenesis. Duplicate culture dishes (diameter, 100 mm) containing the appropriate FBT medium were inoculated with 5×10^5 cells. After 24 h, the dishes were rinsed with phosphate-buffered saline (0.14 M NaCl–2.7 mM KCl–1.5 mM KH_2PO_4–6.5 mM Na_2HPO_4–0.9 mM $CaCl_2$–0.5 mM $MgCl_2$), and the FBT medium was replaced with DMEM supplemented with 10 μM dThd. The cells were allowed to express mutations in this nonselective medium for either 4 days for Sgu resistance or 2 days for Oua resistance. For the selection of Sgu-resistant mutants, ten culture dishes (diameter, 100 mm) containing DMEM supplemented with Sgu were inoculated with 10^5 cells. For the selection of Oua-resistant mutants, five culture dishes (diameter, 100 mm), containing DMEM supplemented with Oua, were inoculated with 5×10^5 cells. The plating efficiency of the cells was determined by inoculating three culture dishes (diameter, 60 mm), containing DMEM, with 10^2 cells. After 10 to 12 days, the dishes were stained and colonies of 50 cells or more were counted. The mutation frequencies were expressed as resistant colonies per 10^5 cells, after correction for the plating efficiencies in the absence of the selective agent.

Protocol for DNA-dependent mutagenesis. Duplicate culture dishes (diameter, 100 mm), containing the appropriate

FBTdC medium, were inoculated with 10^5 cells. After 4 days of growth during which time the DNA was allowed to incorporate BrUra under nonmutagenic conditions, the dishes were rinsed with phosphate-buffered saline, and the FBTdC medium was replaced with DMEM supplemented with 10 μM dThd. After 3 h, the cells were trypsinized and replated under the DNA-dependent conditions. Duplicate culture dishes (diameter, 100 mm), containing DMEM supplemented with either 10 μM dThd or 1 mM dThd plus 100 μM dCyd, were inoculated with 5×10^5 cells. After 3 days of growth, the cells were trypsinized, and 5×10^5 cells were replated, to allow the expression of mutations, into duplicate culture dishes (diameter, 100 mm) containing DMEM. The expression and selection of mutants were performed as described above for the pool-dependent protocol.

Measurement of deoxyribonucleoside triphosphate pools. To determine the levels of deoxyribonucleoside triphosphate (dNTP) pools, exponentially growing cells were washed with ice-cold phosphate-buffered saline and then extracted with 0.4 N $HClO_4$ for 30 min at 4°C. The acid-soluble extracts were neutralized by extraction with an equal volume of 0.5 M tri-*n*-octylamine in trifluorotrichloroethane as described by Khym (15). It was necessary to remove the more abundant ribonucleotides, and this was accomplished by the method of Garrett and Santi (10), which destroys ribonucleotides by the action of periodate and methylamine. To 1.0 ml of neutralized cell extract, 40 μl of 0.5 M $NaIO_4$ was added, followed by 50 μl of 4 M methylamine phosphate, pH 7.5. After 30 min at 37°C, 10 μl of 1 M rhamnose was added to destroy the remaining periodate, and the samples were analyzed by high-pressure liquid chromatography.

For analysis by high-pressure liquid chromatography, a Beckman model 334 liquid chromatograph was used. Aliquots of periodate-treated samples were injected onto an Altex Ultrasil AX anion exchange column (4.6 by 250 mm) and eluted with a linear gradient from 0.2 M ammonium phosphate (pH 3.2) to 0.6 M ammonium phosphate (pH 4.4) at a flow rate of 2 ml/min for 30 min. The column eluate was monitored simultaneously at 254 and 280 nm with an Altex dual-wavelength column monitor. The dNTPs were identified by their retention times and their spectral ratios at these wavelengths. Concentrations were determined by comparison of peak heights with those of accurately prepared standard solutions of approximately the same concentration.

RESULTS

Pool-dependent mutagenesis. A pool-dependent mutagenesis protocol, involving exposure of cells to culture medium that allows the BrdUrd concentration in the medium and the level of BrUra substituted for Thy in DNA to be varied independently, is shown in Fig. 1A. The FBT medium contains BrdUrd, dThd, and FdUrd, an inhibitor of de novo thymidylate biosynthesis (6). Under these culture conditions, the ratio of BrUra to Thy residues in newly synthesized DNA was determined by the ratio of BrdUrd to dThd in the culture medium (13). In this medium, the substitution of BrUra for Thy residues in DNA could be varied at a constant BrdUrd concentration by varying only the dThd concentration. Conversely, the level of BrUra substituted for Thy could be held constant while the BrdUrd concentration in the medium was varied, by maintaining a constant ratio of BrdUrd to dThd. This protocol was used to confirm the relationship between BrdUrd concentration and mutagenesis previously described (13) and to provide a basis for comparing pool-dependent mutagenesis with DNA-dependent mutagenesis. In addition, this protocol was used to determine the mechanism by which deoxyguanosine (dGuo) stimulates BrdUrd mutagenesis, a phenomenon previously described (14) but not elucidated.

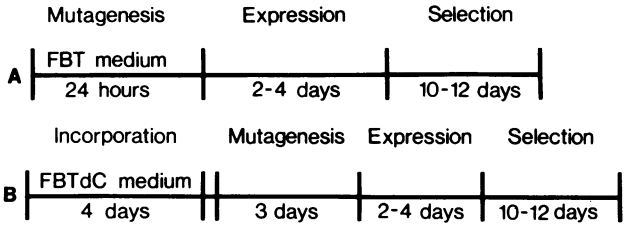

FIG. 1. Bromodeoxyuridine mutagenesis protocols. Details of the protocols are described in the text. (A) Pool-dependent protocol; (B) DNA-dependent protocol.

Syrian hamster cells were grown in FBT medium and then washed and allowed to express any induced mutations before being placed into selection, as described above. When the cells were exposed to low concentrations of BrdUrd in FBT medium, there was no increase in the frequency of cells resistant to the toxic effects of Sgu above that found in control cultures that were not exposed to BrdUrd (Table 1). However, a threefold increase in the BrdUrd concentration, to 50 μM, resulted in a greater than 200-fold increase in mutation frequency. Because the ratio of BrdUrd to dThd in the culture medium was 1.0 at all concentrations, there was no change in the level of substitution of BrUra for Thy in the DNA at the various concentrations (data not shown).

In addition to determining the mutation frequency induced by pool-dependent mutagenesis, the effects of FBT medium on the intracellular dNTP pools were determined. The Syrian hamster cells were exposed to FBT medium in parallel with the mutagenesis experiments, and the dNTPs were measured. The increased mutation frequency induced by increasing BrdUrd concentration correlated with an increase in the ratio of BrdUTP to dCTP (Table 1). This increase was caused by an increase in the size of the BrdUTP pools with increasing BrdUrd concentration, as well as a decrease in the dCTP pool, confirming previous findings (2).

In further pool-dependent experiments, the effects of adding dGuo to FBT medium were determined. The purine deoxyribonucleosides had previously been shown to stimulate BrdUrd mutagenesis, and it was suggested that they acted by perturbing dCyd metabolism (14). When 300 μM dGuo was added to FBT medium containing 15 μM BrdUrd (a nonmutagenic concentration), the mutation frequency was stimulated ca. 30-fold above background levels (Table 2). The ratio of BrdUTP to dCTP increased due to a decrease in dCTP levels. The decreased dCTP levels were caused by increased levels of dGTP, which are known to inhibit the ribonucleotide reductase catalyzed reduction of CDP to dCDP (17). The addition of dCyd to the medium caused an increase in the dCTP levels via the salvage pathways and also suppressed the stimulation of mutagenesis by dGuo. Thus, the stimulation of pool-dependent mutagenesis by dGuo appears to be due to changes in the intracellular ratio of BrdUTP to dCTP.

DNA-dependent mutagenesis. The previous observation that the level of substitution of BrUra for Thy in DNA does not influence the mutation frequency (13) suggested that mutations caused by the replication of BrUra residues in DNA do not play an important role in BrdUrd mutagenesis

TABLE 1. Pool-dependent mutagenesis and dNTP pools[a]

Concn (μM) of medium additive:		Mutation frequency[b]	dNTP[c] (pmol/10^6 cells)					Ratio (BrdUTP/dCTP)
BrdUrd	dThd		dCTP	BrdUTP	dTTP	dATP	dGTP	
	30	0.3	21		63	16	11	
15	15	0.3	23	29	29	15	15	1.3
20	20	2.5	18	43	48	14	13	2.4
30	30	33	6	67	66	16	15	11.2
50	50	194	4	103	107	16	19	25.8

[a] 2E cells were grown in the presence of DMEM containing the indicated additives for 24 h. Duplicate cultures were then processed to determine the induced mutation frequency and the dNTP pools, as described in the text.
[b] Induced mutation frequency is expressed as Sgu-resistant colonies per 10^5 cells after correcting for plating efficiency in the absence of Sgu.
[c] Values shown are the averages of two to four determinations each.

in mammalian cells. However, the nucleotide precursor pool imbalance demonstrated to be required for pool-dependent mutagenesis suggested that the unbalanced precursor environment necessary to induce the mispairing of BrUra residues may not have been present. By analogy, a high ratio of dGTP to dATP might serve to increase the probability of dGTP mispairing with BrUra residues in replicating DNA. A protocol designed to induce DNA-dependent mutagenesis (i.e, mutagenesis caused by BrUra residues in DNA in the absence of BrdUrd in the culture medium and BrdUTP in the intracellular nucleotide pools) is shown in Fig. 1B.

The DNA-dependent protocol entailed the growth of cells under nonmutagenic conditions to uniformly incorporate BrUra into their DNA. This was accomplished through the use of FBTdC medium (see above), which utilizes both dCyd and low concentrations of BrdUrd to prevent the occurrence of any pool-dependent mutagenesis during the incorporation of BrUra into DNA (7, 13). The ratio of BrdUrd to dThd in the FBTdC medium was varied at a constant BrdUrd concentration to control the level of substitution of BrUra for Thy in the DNA. After 4 days of growth in FBTdC medium, the cells, which had incorporated BrUra into their DNA, were washed free of BrdUrd and provided with fresh culture medium containing 10 μM dThd (a source of dThd is necessary for cells which have been exposed to 5-fluorodeoxyuridine). A 3-h incubation in this medium allowed for the clearing of all of the BrdUTP in the nucleotide pools (data not shown). The cells were then transferred to the DNA-dependent conditions. The cells were allowed to replicate their BrUra-containing DNA in the DNA-dependent medium (i.e., no BrdUrd present in the medium) for 3 days, were given time for expression, and were then selected.

In the DNA-dependent mutagenesis experiments, control cultures were exposed to DNA-dependent medium containing a low concentration (10 μM) of dThd (Fig. 2). It can be seen that there was no increase in mutation frequencies above background over the range of 0 to 75% substitution of BrUra for Thy. This was true for the induction of both Sgu resistance and Oua resistance. This result was in agreement with our previous results (13) which indicated that BrUra in DNA in place of Thy was not mutagenic in mammalian cells. However, when the dThd concentration in the DNA-dependent medium was increased to 1 mM (plus 100 μM dCyd to prevent toxicity due to high dThd), very large increases in the mutation frequencies were induced. Furthermore, the mutation frequencies were found to be proportional to the level of BrUra in the DNA. This is the first direct demonstration of DNA-dependent mutagenesis in mammalian cells, presumably resulting from mispairing of BrUra previously incorporated into DNA.

In addition to determining the mutation frequencies induced by DNA-dependent mutagenesis, the effects of the various DNA-dependent conditions on the intracellular dNTP pools were determined. Cells were exposed to the various DNA-dependent conditions for 24 h and then the dNTP pools were measured. The data presented are for cells with no BrUra in their DNA, but similar results were obtained with cells having various amounts of BrUra incorporated into their DNA (data not shown). Exposure to 10 μM dThd had little effect on dNTP levels and therefore on the ratio of dGTP to dATP (Table 3). In controls without added nucleoside, the dGTP-to-dATP ratio was 0.4, whereas in the presence of 10 μM dThd, it was 0.6, not significantly different. However, the presence of 1 mM dThd (plus 100 μM dCyd) caused a large increase in the level of dTTP, which in turn caused a significant increase in dGTP levels. (It has been shown that dTTP is a positive effector of the ribonucleotide reductase-catalyzed reduction of GDP to

TABLE 2. Modulation of pool-dependent mutagenesis by dGuo[a]

Concn (μM) of medium additive:				Mutation frequency[b]	dNTP[c] (pmol/10^6 cells)					Ratio (BrdUTP/dCTP)
BrdUrd	dThd	dGuo	dCyd		dCTP	BrdUTP	dTTP	dATP	dGTP	
	30			0.2	12		43	15	12	
15	15			0.5	11	24	23	17	12	2.1
50	50			201	4	75	72	20	15	18
50	50		100	1.5	34	84	82	21	19	2.5
	30	300		0.9	1		49	17	60	
15	15	300		26	2	28	26	18	54	14
15	15	300	100	0.2	30	30	28	17	60	1.0

[a] 2E cells were grown in the presence of DMEM containing the indicated additives for 24 h. Duplicate cultures were then processed to determine the induced mutation frequency and the dNTP pools, as described in the text.
[b] The induced mutation frequency is expressed as Sgu-resistant colonies per 10^5 cells after correcting for plating efficiency in the absence of Sgu.
[c] Values shown are the averages of two to four determinations each.

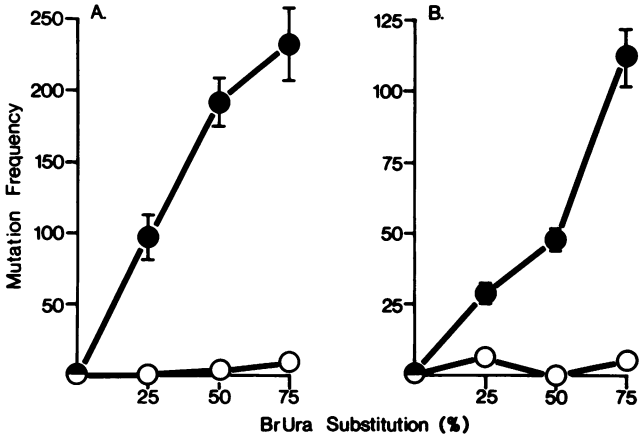

FIG. 2. DNA-dependent mutagenesis in Syrian hamster cells. On the abscissas (A and B), BrUra substitution is expressed as the percentage of Thy residues in DNA that is replaced by BrUra. (A) On the ordinate, the mutation frequency is expressed as Sgu-resistant colonies per 10^5 cells after correction for plating efficiency in the absence of Sgu; (B) on the ordinate, the mutation frequency is expressed as Oua-resistant colonies per 10^5 cells after correction for plating efficiency in the absence of Oua. The data shown are the average of two determinations; the error bar indicates the standard deviation. ○, Cells grown in medium containing 10 μM dThd; ●, cells grown in medium containing 1 mM dThd plus 100 μM dCyd.

dGDP [24].) Although the major effect on the dNTP pools was the increased levels of dTTP, a sevenfold increase in the ratio of dGTP to dATP was observed due to an increase in dGTP levels without any significant change in dATP levels.

In other DNA-dependent mutagenesis experiments (not shown), high concentrations of dGuo were added to the DNA-dependent medium in place of dThd to induce mutagenesis. When the cells were exposed to 1 mM dGuo, a 10-fold increase in the dGTP-to-dATP ratio was observed. However, this increased ratio was due to increases in both the dGTP and the dATP pools. Under these conditions, no increase in the frequency of induced mutations was observed.

Time course of DNA-dependent mutagenesis. Presumably, mutations caused by the mispairing of BrUra residues in replicating DNA need not always occur at the first replication after incorporation of the analog but could continue to be induced as long as BrUra residues are being replicated. This possibility was tested by replacing 50% of the Thy residues in the DNA of Syrian hamster cells with BrUra and exposing the cells to mutagenic, DNA-dependent conditions (1 mM dThd plus 100 μM dCyd) at various times after the removal of BrdUrd from the culture medium. The standard 3-day DNA-dependent period was divided into three 24-h segments (Table 4). The cells containing BrUra in their DNA were allowed to replicate in DMEM containing either 10 μM dThd (nonmutagenic) or 1 mM dThd plus 100 μM dCyd (mutagenic) for the first 24-h period. The subsequent shifting of cells to and from mutagenic and nonmutagenic conditions allowed for the determination of the level of mutagenesis occurring in each of the three 24-h time periods. The data (Table 4) indicate that mutations were induced in each of the three 24-h periods, with a majority occurring in the first 24 h, somewhat fewer occurring in period 2, and the fewest occurring in period 3. The mutation frequencies for the three periods appeared to be additive, giving approximately the frequency induced during 3 days of growth under mutagenic conditions. It is not clear why the level of mutagenesis observed during 24-h period 3 was so low. These results confirm the prediction that BrUra residues in DNA can be mutagenic beyond the first round of DNA replication.

TABLE 4. Time course of DNA-dependent mutagenesis[a]

dThd concn (μM) on day:			Mutation frequency[b]
1	2	3	
10	10	10	0.5
10	10	1,000	8.5
10	1,000	1,000	76
1,000	1,000	1,000	243
1,000	1,000	10	228
1,000	10	10	140

[a] 2E cells were grown in FBTdC medium containing 10 μM BrdUrd and 10 μM dThd for 4 days as described in the text. This resulted in replacement of 50% of the Thy residues in DNA with BrUra.

[b] The induced mutation frequency is expressed as Sgu-resistant colonies per 10^5 cells after correcting for plating efficiency in the absence of Sgu.

DISCUSSION

This report describes a new protocol for BrdUrd mutagenesis in mammalian cells, directly demonstrating the induction of mutations during the replication of BrUra-containing DNA. This mutagenesis involves the incorporation of BrUra into DNA in place of Thy under nonmutagenic conditions, the subsequent removal of BrdUrd from the medium and of BrdUTP from the nucleotide pools, and the replication of the BrUra-containing DNA under conditions of nucleotide pool imbalance. This protocol was shown to induce mutagenesis that is proportional to the level of BrUra in the DNA.

The model proposed by Freese 25 years ago described two mechanisms by which BrdUrd could mispair during DNA replication and act as a mutagen: errors of incorporation (mispairing of BrdUTP) and errors of replication (mispairing of BrUra residues) (9). Previous work from this laboratory (7, 8, 13, 14) provided strong evidence that BrdUrd mutagenesis in mammalian cells occurred primarily by the mispairing of BrdUTP during DNA replication. Because there was no

TABLE 3. DNA-dependent mutagenesis and dNTP pools[a]

Concn (μM) of medium additive:		dNTP[b] (pmol/10^6 cells)				Ratio (dGTP/dATP)
dThd	dCyd	dCTP	dTTP	dATP	dGTP	
		14	19	22	9	0.4
10		20	68	27	15	0.6
1,000	100	37	758	30	84	2.8

[a] 2E cells were grown in the presence of DMEM containing the indicated additives for 24 h. Cultures were then processed to determine the dNTP pools as described in the text.

[b] Values shown are the averages of two to four determinations each.

correlation between mutagenesis and the level of BrUra in DNA in place of Thy, we concluded that the mispairing of BrUra residues during DNA replication was not occurring. These previous results were in contrast to those found in procaryotic systems, for which, over the years, evidence has been put forth for the occurrence of both errors of incorporation (5, 19) and errors of replication (11, 21). The DNA-dependent mutagenesis protocol described in this report clearly demonstrates the occurrence of mispairing due to BrUra residues in DNA in mammalian cells. The experiments presented here satisfy the predictions for such mispairing events: (i) mutagenesis is dependent upon and proportional to the level of BrUra incorporated in place of Thy in the replicating DNA; (ii) mutagenesis occurs when there is no BrdUrd in the culture medium nor BrdUTP in the intracellular pools; and (iii) mutagenesis does not only occur during the first round of replication of BrUra-containing DNA but continues to take place during later rounds as long as BrUra residues are still present.

The mechanism we proposed previously for pool-dependent BrdUrd mutagenesis (2) involved a high ratio of BrdUTP to dCTP in the intracellular nucleotide pools. One might hypothesize that this nucleotide pool imbalance would increase the probability of incorporating BrdUTP opposite a replicating Gua residue. A more general form of such a hypothesis would suggest that a high ratio of incorrect to correct dNTP precursor would be necessary to induce mutagenesis whether the BrdUrd was present as a nucleoside triphosphate or as a residue in replicating DNA. The hypothesis that a high ratio of incorrect to correct dNTP precursor is necessary to induce a Gua-BrUra mispair suggests that the dNTP pool imbalance necessary to induce the mispairing of BrUra residues is not present during pool-dependent mutagenesis. By analogy, therefore, a high intracellular ratio of dGTP to dATP (incorrect to correct dNTP) might increase the probability of mispairing dGTP with a BrUra residue in replicating DNA. The DNA-dependent mutagenesis protocol described above was shown to generate a high dGTP-to-dATP-ratio environment for the replication of BrUra-containing DNA. This environment was attained by adding high concentrations of dThd to the culture medium. This caused increased dTTP pools, which are known to stimulate the ribonucleotide reductase-catalyzed reduction of GDP to dGDP (24), leading to higher dGTP levels and therefore an increased dGTP-to-dATP ratio. Although the results are consistent with the hypothesis that the increased dGTP-to-dATP ratio increased the probability of dGTP mispairing with replicating BrUra residues, the possibilty that the increased dTTP levels have a more direct involvement (e.g., mispairing with BrUra) cannot be ruled out at this time. Recent findings (R. S. Lasken and M. F. Goodman, J. Biol. Chem., in press), utilizing an in vitro DNA synthesis system, indicate that BrUra-Gua mispairs do occur as a result of misincorporation of BrdUTP during DNA synthesis on a synthetic copolymer template containing Gua residues but no adenine residues. However, when BrUra residues were in the template and the misincorporation of dGTP was measured, data consistent with other types of mispairs as well as BrUra-Gua mispairs were also obtained.

The Freese model for BrdUrd mutagenesis predicts that errors of incorporation should only induce GC-to-AT transitions and that errors of replication should only induce AT-to-GC transitions. The two protocols for BrdUrd mutagenesis described in this report may therefore induce different and reciprocal base substitution events (i.e., pool-dependent mutagenesis might induce GC-to-AT transitions, whereas DNA-dependent mutagensis might induce AT-to-GC transitions) in mammalian cells. Experiments designed to determine the molecular mechanism of mutagenesis for each protocol are currently underway. Preliminary results (unpublished data) indicate that each protocol does have a high level of specificity as to the mechanism by which mutations are induced and that the two protocols appear to involve different and somewhat reciprocal base substitutions (i.e., mutants generated by pool-dependent mutagenesis were found to be more efficiently reverted by the DNA-dependent protocol than by the pool-dependent protocol). This system appears to be unique in that simply by changing the experimental protocol, one can generate highly specific but different mutagenic events with the same mutagen.

ACKNOWLEDGMENTS

I thank Pamela Richards for her excellent technical assistance and Linda Cardenas for her excellent secretarial help in preparing the manuscript.

This work was supported by Public Health Service grants CA31777 and CA31781 from the National Cancer Institute.

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