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In Vivo Replication of Hepatic Deoxyribonucleic Acid of Rats Treated with Dimethylnitrosamine: Presence of Dimethylnitrosamine-Induced O^6-Methylguanine, N^7-Methylguanine, and N^3-Methyladenine in the Replicated Hybrid Deoxyribonucleic Acid†

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ABSTRACT: Experiments were designed to determine whether some chemical lesions such as O^6-methylguanine, N^7-methylguanine, and N^3-methyladenine induced in rat liver DNA by the hepatocarcinogen dimethylnitrosamine permit replication in vivo. For this purpose, [^{14}C]dimethylnitrosamine was administered to methylate the parental strand of liver DNA. Four hours later, a time period when the carcinogen cannot be detected in either the liver or the blood, rats were subjected to partial hepatectomy in order to induce DNA replication. During the S phase, 5-bromo-2-deoxyuridine was administered to render the newly made strands heavy. The rebanded, hybrid, hepatic DNA of density 1.714 g/cm^3 and greater was pooled from the neutral cesium chloride gradient, dialyzed, and lyophilized. The hybrid DNA was then treated with S_1 nuclease to digest any single-stranded regions. The results obtained indicated the presence of O^6-methylguanine, N^7-methylguanine, and N^3-methyladenine in S_1 nuclease resistant, hybrid DNA. The results are interpreted to indicate that these chemical lesions permitted in vivo DNA replication.

Cell proliferation has often been implicated in cell transformation and the development of preneoplastic and neoplastic lesions with chemicals, radiation, and viruses (Todaro & Green, 1966; Borek & Sachs, 1968; Pound, 1968; Warwick, 1971; Rajewsky, 1972; Kakunaga, 1974; Rajalakshmi & Sarma, 1975; Craddock, 1976; Cayama et al., 1978; Ying & Sarma, 1979). Although the mechanism by which cell proliferation stimulates the induction of preneoplastic and neoplastic cell populations is not known, replication of carcinogen-damaged DNA prior to repair offers an attractive mechanism by which carcinogen-induced lesions in DNA can become fixed in the newly made DNA (Rajalakshmi & Sarma, 1975; Sarma et al., 1975; Craddock & Henderson, 1978). It is therefore important to determine whether DNA with carcinogen-induced lesions replicates in vivo. We had earlier shown that hepatic DNA damaged by liver carcinogens DMN[1] and *N*-hydroxy-2-(acetylamino)fluorene does replicate in vivo, generating stable DNA of normal size even though the parental strand had alkali-sensitive lesions, as measured by sedimentation analysis using alkaline sucrose gradients (Rajalakshmi & Sarma, 1975; Zahner et al., 1977). These results indicated that carcinogen-induced alkali-sensitive lesions in DNA permit DNA replication in vivo. However, since the interaction of carcinogens with DNA results in several chemical lesions (Irving, 1973; Sarma et al., 1975; Magee et al., 1975; Singer, 1975; Pegg, 1977; Miller, 1978), the question arises whether all lesions permit replication and, if they do, with what efficiency. In the current investigation, we have attempted to determine whether some specific methylated bases induced in liver DNA by DMN will permit replication of the DNA in vivo.

The experimental approach consisted of (a) labeling the chemical lesions in the parental strand of DNA with [^{14}C]-DMN, (b) inducing cell proliferation to stimulate replication

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[1] Abbreviations used: DMN, dimethylnitrosamine; m^{7}G, N^7-methylguanine; m^{6}G, O^6-methylguanine; m^{3}A, N^3-methyladenine; BrdUrd, 5-bromo-2-deoxyuridine; CsCl, cesium chloride; EDTA, tetrasodium ethylenediaminetetraacetate; PCA, perchloric acid.

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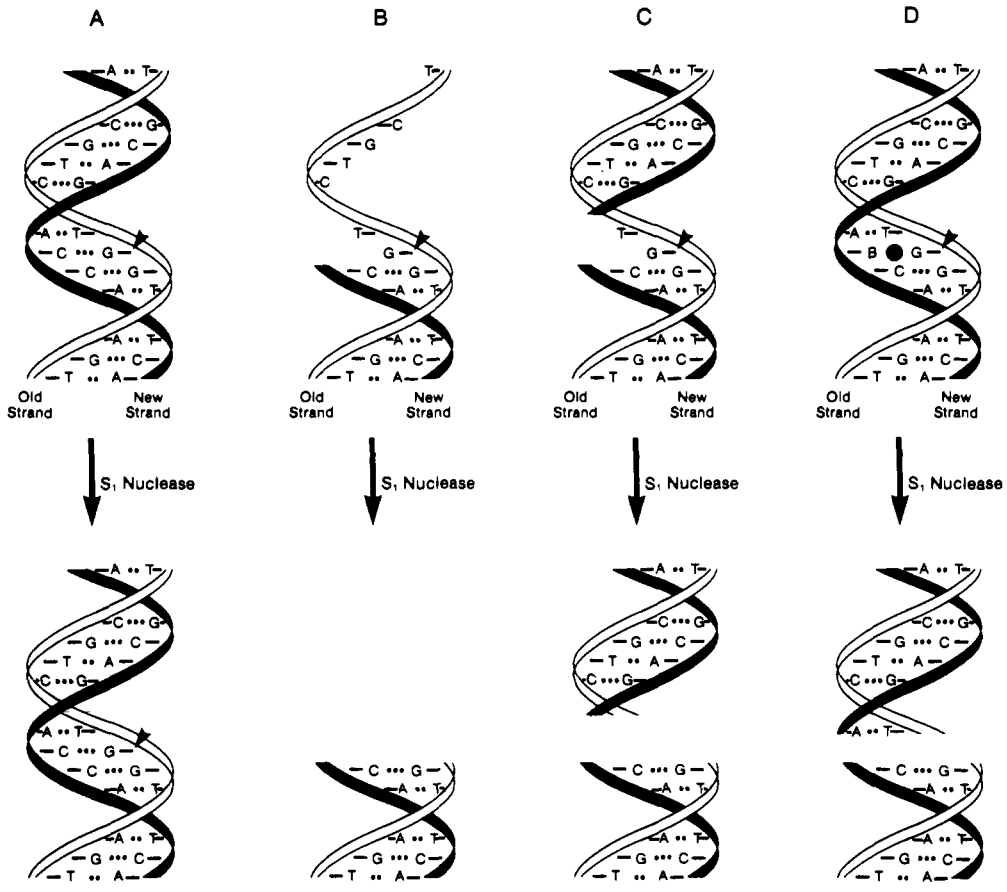

FIGURE 1: Representation of some possible patterns of the hybrid DNA before and after S_1 nuclease treatment. (▶) represents the carcinogen-induced lesion in the DNA. (A) DMN-induced lesions in DNA permit replication, hence the hybrid DNA becomes S_1 nuclease resistant. (B and C) DMN-induced lesions do not permit replication, hence the imperfect hybrid DNA becomes S_1 nuclease susceptible; (B) represents the hybrid with external single-strand regions and (C) represents the hybrid with internal single-strand regions. (D) represents DMN-induced lesions that permit replication but because of imperfect complimentarity (denoted by B●G) the hybrid DNA becomes S_1 nuclease susceptible.

of the damaged DNA, (c) rendering the DNA of the daughter strand heavy by density labeling with BrdUrd, (d) separating the replicated hybrid DNA by isopycnic centrifugation on neutral CsCl gradients, (e) treating the hybrid DNA with S_1 nuclease to digest the nonreplicated parental strand DNA in the hybrid (Figure 1), and (f) characterizing the methylated bases present in the S_1 nuclease resistant DNA. It is assumed that the S_1 nuclease resistant hybrid DNA is the newly replicated DNA and the methylated bases present in this hybrid represent the chemical lesions in the parental strand that permitted replication.

The results of the present study suggest that m^7G, m^6G, and m^3A in liver DNA induced by DMN permitted replication in vivo.

Materials and Methods

White male Wistar (Charles River) rats, weighing 130–160 g, were used in all experiments. The animals had free access to water and food. DMN was obtained from K & K Laboratories and Aldrich Chemicals. [^{14}C]DMN (sp act. 50 mCi/mmol), [^{3}H]DMN (sp act. 100 mCi/mmol), and [*methyl*-^{3}H]thymidine (sp act. 55.56 Ci/mmol) were purchased from New England Nuclear. BrdUrd and CsCl were from Sigma Chemical Co. and Harshaw Chemicals, respectively. Sephadex G-10 was obtained from Pharmacia. Pronase was obtained from Calbiochem and RNase (bovine pancreas) was obtained from P-L Biochemicals, Inc. S_1 nuclease (*Aspergillus oryzae*) was obtained from Miles Laboratories, Inc. Cellulose nitrate tubes (5/8 × 3 in.) (Beckman Instruments, Inc.) and dialysis tubing (A. H. Thomas) were treated with a 0.5% boiling solution of EDTA (pH 7.5) for 10 min, rinsed with distilled water, and dried before use.

Isolation of S_1 Nuclease Resistant Hybrid DNA Containing [^{14}C]DMN-Induced Methylated Bases. Rats were injected intraperitoneally with [^{14}C]DMN [(100 μCi/0.5 mg)/100 g of body weight]. Administration of this dose of DMN neither delayed the onset of S phase nor inhibited DNA synthesis to any significant extent (Rajalakshmi & Sarma, 1975). Four hours following the administration of DMN, a time period when all the free DMN disappeared from both plasma (Abanobi et al., 1977) and liver (Ying & Sarma, 1979), the animals were subjected to 67% partial hepatectomy (Higgins & Anderson, 1931) to induce DNA replication. During the S phase, BrdUrd (7 mg/100 g of body weight) was injected 6 times, every 2 h starting 16 h after partial hepatectomy, to render the newly made strands heavy. Rats were killed 18 h after the last injection, a time period when the newly made DNA is greater than 10^9 daltons (Rajalakshmi & Sarma, 1975), and the liver chromatin was prepared. All steps from the time the animals were killed until the preparation of S_1 nuclease resistant DNA were carried out under red light.

Isolation of Chromatin. Hepatic chromatin was prepared by the method described by Rajalakshmi et al. (1978). The procedure consists of (1) homogenization of the liver in 0.075 M NaCl, 0.025 M EDTA (pH 7.6), and 0.1% Triton X-100, using a Potter-Elvehjem homogenizer fitted with a Teflon pestle, (2) centrifugation of the homogenate after an interval of 10 min at 3000 rpm for 15 min in an International Model PR6 refrigerated centrifuge, (3) washing of the nuclear pellet 3 times with 0.05 M Tris-HCl (pH 7.5) containing 0.1% Triton

X-100, followed by three more washings with 0.05 M Tris-HCl (pH 7.6) and finally one washing with 0.01 M glycine (pH 6.0), and (4) solubilization of chromatin in 0.01 M glycine, 16 mL/g of liver, overnight. A clear chromatin solution was obtained by gentle homogenization before use.

Purification of DNA. Rat liver chromatin DNA was deproteinized with autodigested Pronase (100 μg/mL) in the presence of 0.01 M Tris-HCl (pH 7.6), 0.05% sodium dodecyl sulfate, and 1 M NaCl at 37 °C for 4 h and further purified by Marmur's (1961) procedure. DNA was precipitated with ethanol and spooled. The spooled DNA was redissolved in 0.01 M NaCl solution (200–300 μg of DNA per mL) and treated with RNase (100 μg/mL). After an incubation period of 2 h at 37 °C, the pure DNA was isolated, as described above.

Fractionation of DNA by Isopycnic Neutral CsCl Gradient Centrifugation. DNA was dissolved in 0.01 M NaCl. CsCl was added to the DNA solution, to an initial density of 1.706 g/cm^3. Five milliliters of the solution containing a maximum of 400 μg of DNA was added to EDTA-pretreated cellulose nitrate tubes, and the tubes were filled with mineral oil. Centrifugation was carried out in a Spinco 50Ti rotor at 33 000 rpm for 60 h at 20 °C. The gradients were decelerated without the brake and were fractionated. The fractions were monitored for A_{260nm}, acid-precipitable radioactivity, and the refractive index. Fractions of density 1.714 g/cm^3 and greater were pooled and rebanded by centrifugation at 33 000 rpm for 60 h at 20 °C. DNA in fractions of density of 1.714 g/cm^3 and greater were pooled and dialyzed for 16–20 h at 4 °C (3 times against distilled water; once against 0.01 M NaCl). The DNA was lyophilized and stored at −20 °C until use.

In some experiments, Triton X-100 washed nuclei were suspended in a solution containing 1% sodium dodecyl sulfate, pH 7.0, 1.4 M NaCl, and 0.01 M EDTA. CsCl (1.26 g/mL) was added to the nuclear suspension and centrifuged at 10000*g* for 15 min at 10 °C. The clear solution below the protein layer was adjusted precisely to an initial density of 1.706 g/cm^3 and centrifuged for 60 h at 33 000 rpm. DNA was prepared from fractions of density 1.714 g/cm^3 and greater as described.

Digestion of Methylated Hybrid DNA by S_1 Nuclease. The DNA solution in 0.01 M NaCl was incubated with S_1 nuclease at a concentration of 40 μg/50 μg of DNA in a reaction mixture containing 0.04 M sodium acetate, pH 5.0, 0.15 M sodium chloride, and 2 mM zinc chloride. After an incubation period of 1 h at 37 °C, the reaction mixture was chilled on ice, and bovine serum albumin (40 μg/mL) and 1 N PCA (to a final concentration of 0.5 N) were added. After 15 min on ice, the samples were centrifuged at 4000 rpm for 15 min at 5 °C in an International Model PR6 centrifuge.

Fractionation of Methylated Purines. The S_1 nuclease resistant, methylated DNA was hydrolyzed with 0.1 N PCA, either at 37 °C for 16 h or at 70 °C for 45 min, and the methylated bases were fractionated on Sephadex G-10 columns by using 0.05 M ammonium formate (pH 6.8) as the eluting buffer (Lawley & Shah, 1972). m^{7}G, m^{6}G, and m^{3}A were identified by using authentic standards as markers. The purity of the methylated bases was checked by paper chromatography, using different solvent systems (Rao et al., 1978). Pooled fractions were lyophilized, and the radioactivity was determined by using an Intertechnique scintillation spectrometer.

Results

Initial experiments were carried out to determine the dose of BrdUrd to be administered which would produce a measurable increase in the density of liver DNA without any obvious toxicity to the animal or inhibitory effects on liver DNA synthesis. In the dose range of 600 μg–120 mg per 100 g of body weight given in 6 equally divided doses, 7 mg/dose was found to shift the peak density of liver DNA from 1.699 g/cm^3 to a peak density of 1.7060 g/cm^3 (Figure 2).

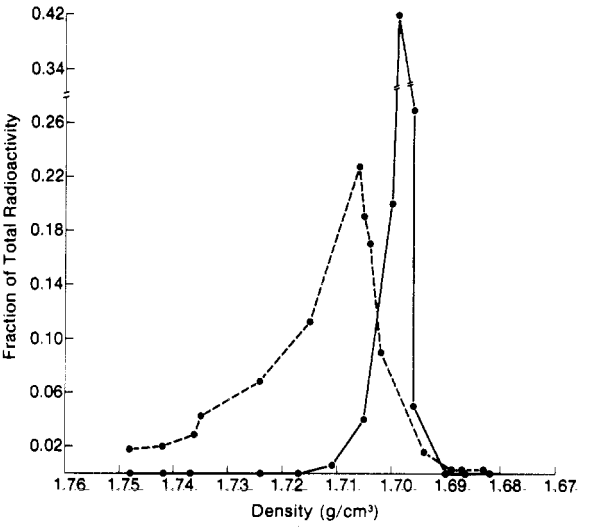

FIGURE 2: Effect of BrdUrd incorporation on the density distribution of replicated rat liver DNA. In each experiment, six rats were injected with [*methyl*-^{3}H]thymidine ip 6 times at 2-h intervals at a dose of 17 μCi/100 g of body weight, beginning 16 h after partial hepatectomy. BrdUrd (7 mg/100 g per dose) or 0.9% NaCl was injected 5 min after each injection of thymidine, and the rats were killed 18 h after the last injection. The purified DNA was centrifuged by using CsCl gradients as described under Materials and Methods. The profiles represent the first banding in CsCl gradients. (●—●) [*methyl*-^{3}H]Thymidine plus 0.9% NaCl; (●---●) [*methyl*-^{3}H]thymidine plus BrdUrd.

In these experiments rat liver DNA was labeled with tritiated thymidine. In order to label the DNA efficiently, we injected [^{3}H]thymidine prior to the administration of BrdUrd; otherwise, the large amounts of the analogue may compete and abolish the incorporation of tritiated thymidine which was given in trace doses.

In Figure 3 is shown the density gradient profile of DNA methylated by [^{14}C]DMN and also substituted with BrdUrd. The peak density of alkylated liver DNA varied from 1.6910 to 1.6990 g/cm^3. Nevertheless, in all experiments, administration of BrdUrd during replication resulted in liver DNA of higher density. Identical density profiles were obtained whether the DNA in the gradient fractions was monitored by measuring either the acid-precipitable radioactivity or absorption at 260 nm.

To establish that replicated DNA hybrids are not contaminated with unreplicated DNA, we performed an analysis of distribution of liver DNA replicated in the presence and absence of BrdUrd treatment. The data in Table I indicate that no DNA which replicated in the absence of BrdUrd banded at or above a density of 1.714 g/cm^3, whereas 21–28% of liver DNA replicated in the presence of BrdUrd banded at and above this density. Hence, only the DNA fractions sedimenting at a density greater than, or equal to, 1.714 g/cm^3 in the first and second (rebanded) neutral CsCl gradients were used to isolate in vivo replicated DNA hybrids containing carcinogen-induced labeled methylated bases and BrdUrd.

In order to free the isolated hybrid DNA of any single-stranded regions and imperfectly paired regions, we treated it with S_1 nuclease, an enzyme that specifically digests single-stranded regions present either internally or at the ends (Figure 1) (Ando, 1966; Shishido & Ando, 1972; Vogt, 1973;

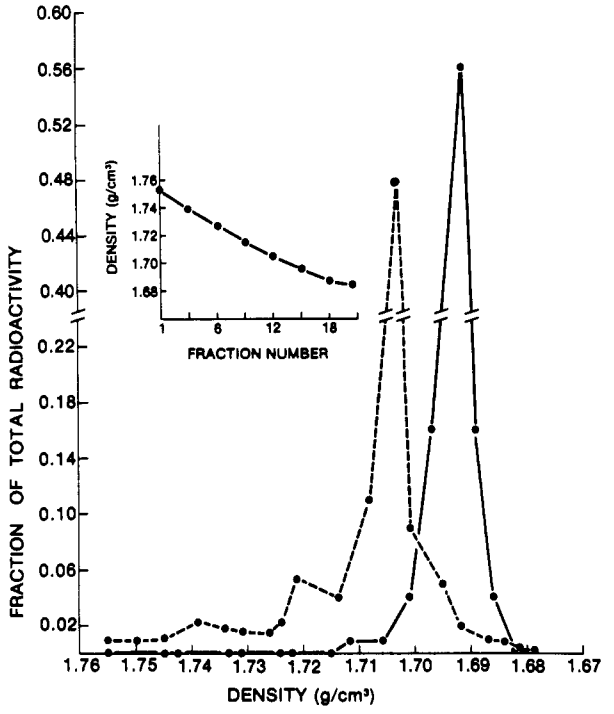

FIGURE 3: Effect of BrdUrd incorporation on the density distribution of replicated rat liver methylated DNA. In each experiment, five rats were injected with [^{14}C]DMN ip at a dose of (0.5 mg/100 μCi)/100 g of body weight, 4 h before partial hepatectomy. Three rats received BrdUrd while the other two received 0.9% NaCl by using the same protocol as described in Figure 2. The profiles represent the first banding in CsCl gradients. (●—●) [^{14}C]DMN plus 0.9% NaCl; (●---●) [^{14}C]DMN plus BrdUrd. The insert represents the density profile of the CsCl gradient.

Table 1: Density Analysis of Liver DNA from Rats Treated with BrdUrd[a]

treatment	% of DNA at $\rho \geq$ 1.691	$\rho \geq$ 1.706	$\rho \geq$ 1.714
[*methyl*-^{3}H] thymidine			
−BrdUrd	99	1	0
+BrdUrd	98	52	28
[^{14}C] dimethylnitrosamine			
−BrdUrd	80	1	0
+BrdUrd	98	32	21

[a] The data using [*methyl*-^{3}H] thymidine are generated from the values presented in Figure 2 and those using [^{14}C] dimethylnitrosamine are generated from the values presented in Figure 3.

Table II: S_1 Nuclease Digestion of Liver DNA of Control Rats and Rats That Received [^{3}H]DMN

DNA	S_1 nuclease	% total dpm rendered PCA soluble at 0 °C
rat liver DNA[a]		
native	−	3
native	+	7
denatured (heat)[b]	−	4
denatured (heat)	+	92
denatured (0.3 N NaOH)[c]	−	3
denatured (0.3 N NaOH)	+	95
DNA methylated by [^{3}H]DMN in vivo		
native	−	20
native	+	32
denatured (pH 12.5)[d]	−	33
denatured (pH 12.5)	+	96

[a] Rat liver DNA labeled with tritiated thymidine was used. [b] DNA was denatured in a boiling-water bath for 15 min and then chilled on ice. [c] DNA was denatured by incubating with 0.3 N NaOH for 15 min at 37 °C and then neutralized. [d] Replicated hybrid DNA of density 1.7212 g/cm^3 (after the first rebanding in neutral CsCl) obtained from DMN-treated rat liver was incubated at pH 12.5 for 15 min at 37 °C and then neutralized. Other details are described under Materials and Methods.

Table III: Distribution of Radioactive Methylated Purines in S_1 Nuclease Resistant Replicated Hybrid Hepatic DNA following the Administration of [^{14}C] DMN[a]

methylated purine	radioact. (pmol/mg of DNA)
N^7-methylguanine	105
O^6-methylguanine	18
N^3-methyladenine	17

[a] The S_1 nuclease resistant, hybrid DNA was hydrolyzed and the methylated bases were fractionated on Sephadex G-10 columns. Pooled fractions were lyophilized and the radioactivity was determined. The experiment was repeated 6 times, 4 times using [^{14}C]-DMN and 2 times using [^{3}H] DMN. The results of a typical experiment are presented. The three methylated bases were present in all preparations of S_1 nuclease resistant, hybrid DNA, although the extent of each varied in the different experiments. For example, m^{7}G varied from 85 to 154 pmol/mg of DNA, m^{6}G varied from 11 to 64 pmol/mg of DNA, and m^{3}A varied from 8 to 26 pmol/mg of DNA.

Ghangas & Wu, 1975; Wiegand et al., 1975). The results in Table II indicate that, under the conditions employed, over 90% of the heat- or alkali-denatured DNA was digested, when monitored by the release of radioactivity. Similar results were obtained when the reaction was monitored by absorption at 260 nm. Less than 4% of the native unmethylated DNA was solubilized by S_1 nuclease. The results also indicate that the replicated, in vivo methylated, denatured rat liver DNA is equally susceptible to S_1 nuclease. The high-acid blanks obtained when either the native or the denatured [^{3}H]DMN methylated DNA was employed may be because of the acid lability of some of the methylated DNA products.

The results presented in Table III clearly indicate the presence of m^{7}G, m^{6}G, and m^{3}A in the S_1 nuclease resistant, replicated, hybrid DNA. A similar pattern of results was obtained when liver chromatin was used instead of purified DNA as the starting material for fractionation of replicated and nonreplicated DNA using neutral CsCl gradients. Similarly, the presence of methylated purines could be demonstrated in the S_1 nuclease resistant, replicated, hybrid DNA when [^{3}H]DMN was used instead of [^{14}C]DMN. The results presented in Table III are, in fact, an underestimate, because, in these experiments, only DNA of density 1.714 g/cm^3 and greater was used for S_1 nuclease digestion. Such a fractionation, although it yields hybrids free of unreplicated DNA, also results in the loss of considerable amounts of replicated DNA (see Table I). In addition, BrdUrd-incorporated DNA is light sensitive and might have acquired S_1 nuclease sensitive sites, in spite of the fact that all the steps were carried out under red light. Experiments are in progress to determine the rate at which these lesions permit in vivo replication.

The high ratio of m^{3}A and m^{6}G to m^{7}G in the replicated hybrid is of interest. Although a definitive explanation cannot be given at present, it is likely that the half-life of m^{3}A and m^{6}G but not of m^{7}G may be longer in the replicating liver. Craddock (1973) has demonstrated that the half-life of m^{3}A

was 7 h in regenerating liver compared to 3 h in nonregenerating liver. In addition, since the sites of carcinogen interaction with DNA are nonrandomly distributed in DNA and the rates of removal of DNA-bound carcinogen from different regions of DNA are not the same (Cooper et al., 1975; Ramanathan et al., 1976a,b; Moyer et al., 1977; Metzger et al., 1977; Sarma et al., 1978; Schwartz & Goodman, 1979), and since only 21% of the liver DNA was anaylzed (see Table I), it is possible that the methylated bases characterized in the present study may constitute a selective distribution in certain segments of DNA that can be fractionated as hybrid DNA.

Discussion

The data presented in this study reveal that the liver DNA of rat treated with the carcinogen DMN replicates in vivo and that the replicated DNA containing the methylated bases m^7G, m^6G, and m^3A can be isolated as a hybrid of density 1.714 g/cm^3 and higher. The two most important assumptions necessary to arrive at this conclusion are (1) the DNA of density 1.714 g/cm^3 or greater represents replicated DNA and is a perfect duplex in nature and (2) the radioactive methylated products present in this hybrid DNA are associated with the parental strand. These assumptions are supported by the following evidence.

The conclusion that the shift in the density is because of replication and not due to a repair process is based on the fact that hybrid DNA such as described in this study can be isolated from the liver only after the DNA synthesis in response to a proliferative stimulus like partial hepatectomy and not from resting liver. Our earlier experiments have shown that neither radioactive thymidine nor BrdUrd can be incorporated into liver DNA to any significant extent after a single ip injection of DMN at doses used in the present study (unpublished observations). In addition, if BrdUrd was to be incorporated into DNA entirely because of the repair process, a shift in the density of DNA as seen in the present experiments would not be noticed (Pettijohn & Hanawalt, 1964).

The methylated bases found in the hybrid DNA are derived mainly from the replicated regions of DNA because nonreplicated regions that may be present in the hybrid would have been removed by S_1 nuclease due to their single-stranded nature (see Figure 1).

The possibility that the methylated bases could be derived from any contaminating RNA or from the alkylation of the daughter strands of DNA by any residual [^{14}C]DMN remaining in circulation was ruled out in view of the following. Since the DNA was prepared from Triton X-100 washed nuclei, it would be free of all cytoplasmic RNA and those ribosomes that are generally found attached to the outer nuclear membrane; further, the purified DNA was treated with RNase. Under the centrifugal conditions employed, RNA would pellet on the walls of the tube and S_1 nuclease treatment would have digested free single-stranded RNA, if present. In addition, the DNA was isolated 48 h after the administration of [^{14}C]DMN, at which time most of the RNA alkylated during its synthesis would have left the nucleus. The possibility of alkylation of daughter-strand DNA by [^{14}C]DMN can be ruled out in view of the following experimental evidence. The differential pulse polarographic technique permits the detection of DMN at concentrations as low as 10 ppm (Chang & Harrington, 1975). By use of this method, measurable levels of DMN could not be detected in the serum of rats 2 h following its administration at a level of 10 mg/kg (Abanobi et al., 1977). DMN in liver was measured by using gas chromatographic and high-pressure liquid chromatographic techniques, wherein the minimum level of detection is 5–10 ng/mL (T. S. Ying, unpublished observations). By use of these methods, DMN could not be detected in the liver at 4 h following the administration of a dose of 5 mg of DMN per kg of body weight. These data thus support the conclusion that the radioactive methylated bases present in the replicated hybrid are probably in the parental strand.

The possibility that segments of parental DNA containing methylated bases are incorporated into the newly made DNA by recombination events is worth considering. Although not unequivocally ruled out, such a possibility appears to be less likely because, under similar experimental conditions, the newly made DNA synthesized in vivo on a DMN alkylated template DNA was free of alkali-sensitive lesions in spite of their presence in the parental strand (Rajalakshmi & Sarma, 1975).

Several lines of evidence indicate that certain types of lesions in DNA induced by UV and carcinogens block DNA synthesis (Rupp & Howard-Flanders, 1968; Lehman, 1972; D'Ambrosio & Setlow, 1976; Caillet-Fauquet et al., 1977; Hsu et al., 1977; Villani et al., 1978; Moore & Strauss, 1979), and the block apparently is at the site of the lesion on the parental strand of the DNA. In procaryotes for example, thymine dimers block DNA chain elongation, creating gaps (Swenson & Setlow, 1966; Rupp & Howard-Flanders, 1968; Hewitt & Gaskins, 1971), and these gaps are filled in eventually by a recombination type of repair (Rupp & Howard-Flanders, 1968; Rupp et al., 1971; Ganesan, 1974). However, in eucaryotes, using cells in culture, the gaps generated across thymine dimers are filled in by de novo synthesis (Lehman, 1972). Bates et al. (1970) had isolated hybrid DNA containing 7,12-dimethylbenz[*a*]anthracene on the parental strand and BrdUrd in the daughter strand of the hybrid. However, the hybrid was not subjected to S_1 nuclease digestion and, hence, it is difficult to conclude whether or not the carcinogen-induced lesion permitted replication.

The present study is the first instance where the presence of carcinogen-induced lesions such as m^7G, m^6G, and m^3A has been demonstrated in the S_1 nuclease resistant, replicated hybrid DNA. The data suggest that these lesions might have permitted replication, thereby increasing the chances of introducing errors, a phenomenon that may play an important role in the initiation of carcinogenesis. Alternatively, a similar pattern of results may also be obtained if the replication was by a mechanism involving strand displacement and branch migration (Higgins et al., 1976), a process that may be error free. The fact that cell proliferation is an important prerequisite in the induction of preneoplastic and neoplastic lesions and cell transformation by chemicals, radiation, and viruses suggests that the replication of carcinogen-damaged DNA may involve an error-prone mechanism.

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