IN VIVO CHAIN ELONGATION OF HEPATIC DNA: 1-β-D-ARABINOFURANOSYL CYTOSINE SENSITIVE AND INSENSITIVE STEPS

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SUMMARY: The in vivo chain elongation of rat liver DNA following partial hepatectomy was studied using alkaline sucrose gradients. DNA made in 5 min was less than 4 x 10^7 daltons and that made in 30 min was heterodisperse and by 4 hr 75% of the DNA became larger than 1 x 10^9 daltons. Administration of 1-β-D-arabinofuranosyl cytosine (ara-C) 5 min after thymidine-^{3}H injeciton inhibited the chain elongation, whereas if given 30 minutes after thymidine-^{3}H pulse did not inhibit the chain elongation. Thus the in vivo chain elongation of rat liver DNA consists of at least two steps 1) a step sensitive to ara-C involving nucleotides addition and 2) the other insensitive to ara-C and probably involving ligation of polynucleotide chains.

INTRODUCTION: During the course of investigations on the replication of DNA damaged by carcinogens, it became necessary to establish the kinetics of hepatic DNA chain elongation in vivo in normal and carcinoge-treated rats. Although DNA synthesis has been investigated in great detail in prokaryotes and to some extent in eukaryotes using cells grown in culture, very little information is available regarding the kinetics of in vivo DNA chain elongation in intact rat liver. In regenerating liver, the appearance of small molecular weight DNA as intermediates was noticed during the early period of DNA synthesis (1,2). However, the DNA used for the analysis of size distribution was isolated and purified by procedures that involved considerable amount of shearing. In the present communication we wish to report hepatic DNA synthesis in vivo using methods that involved minimal shearing. The DNA chain elongation in the liver consists of at least two steps: 1) synthesis of short chains of polydeoxyribonucleotides of low molecular weight sensitive to 1-β-D-arabinofuranosyl cytosine (ara-C) an inhibitor of DNA synthesis (3-7) and 2)

step or steps insensitive to ara-C. These two steps may represent chain elonga tion due to nucleotide addition and ligation respectively.

MATERIALS AND METHODS

White male rats of Wistar strain (Carworth Farms) weighing about 130-150 g were used in all experiments. Thymidine-methyl-^{3}H (sp. activity 20 Ci/mmole) was purchased from New England Nuclear, Boston, Mass. Ara-C was obtained from Sigma Chemical Co., St. Louis, Mo. Partial hepatectomy was carried out according to the procedure of Higgins and Anderson (8). 21 Hours after partial hepatectomy 400 µC of thymidine-methyl-^{3}H was given intraperitoneally to each rat. Five or 30 minutes later either 0.9% NaCl or ara-C (250 mg/kg) in water (adjusted to neutral pH) was injected intraperitoneally. Rats were killed by decapitation 240 minutes after thymidine-methyl-^{3}H administration. The size of the DNA was measured by sedimentation in alkaline sucrose density gradient. The preparation of sucrose gradients, suspension of liver nuclei, lysis of nuclei and release of DNA on the top of sucrose gradient and other technical details were carried out as described earlier (9). The alkaline lysing agent consisted of 0.3 M NaCl; 0.03 M EDTA, 0.1 M Tris-HCl pH 12.5 and 0.5% SDS. In experiments where the specific activity of the DNA was determined, the DNA was isolated and purified by the procedure described by Kirby and Cook (10). DNA was determined by the diphenylamine method of Burton (11). The acid precipitable radioactivity from the sucrose gradient represented DNA, since the radioactivity was found to be completely alkali resistant and DNase hydrolysable.

RESULTS AND DISCUSSION

Each gradient represents one animal and each experiment was repeated nine times. In Fig. 1 are shown typical sedimentation profiles illustrating the kinetics of DNA chain elongation in regenerating liver. At 5 and 30 min following the thymidine-methyl-^{3}H injection, the newly made DNA appears as short chains of low molecular weight in the alkaline sucrose gradient. Approximately 70% of the DNA at 5 min has a molecular weight of less than 4×10^7 daltons,

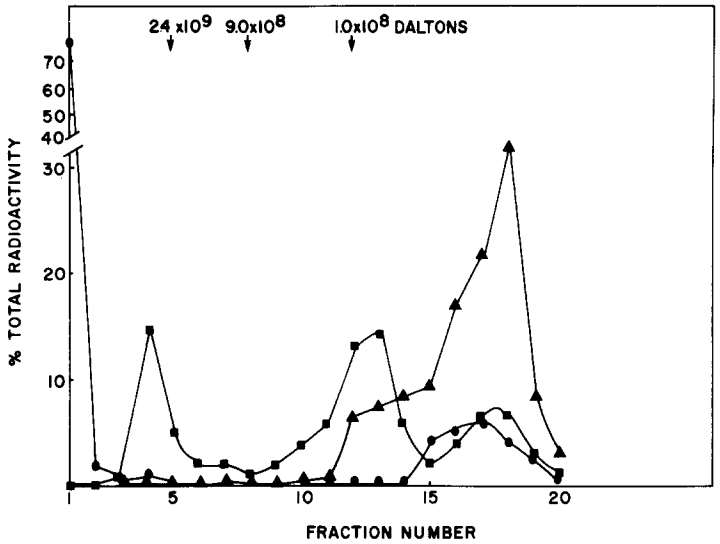

Fig. 1. Kinetics of rat liver DNA chain elongation in vivo: Sedimentation of the newly made DNA in sucrose gradients. Rats were partially hepatectomized. 21 hr. later they were given 400 μc of thymidine-methyl-^{3}H intraperitoneally and were killed at 5 (▲-▲-▲) 30 (■-■-■) or 240 min (●-●-●). Preparation of the alkaline sucrose gradient and of the nuclear suspension of squashed liver, and lysis of such suspension on top of the gradient were described earlier (9). Sedimentation of the DNA is from right to left. Total acid precipitable radioactivity recovered from each gradient was: 5 min pulse 305 cpm; 30 min pulse 791 cpm; 240 min pulse 993 cpm.

17% about 1 x 10^8 daltons (between 4 x 10^7 to 6 x 10^8 daltons) and only 8% larger than 1 x 10^9 daltons. In contrast, at 30 min. 27% is greater than 1 x 10^9 daltons, 42% about 1 x 10^8 daltons (ranging between 4 x 10^7 to 6 x 10^8 daltons) and only 26% smaller than 4 x 10^7 daltons.

The sedimentation profile of the DNA shifts progressively toward increasing molecular weight and by 4 hr nearly 75% of the DNA is larger than 1 x 10^9 daltons. At this time only a small proportion, 10% of about 1 x 10^8 daltons and 14% smaller than 4 x 10^7 daltons, was observed.

These results indicate that in regenerating rat liver, DNA is replicated by a discontinuous mechanism involving the synthesis of nascent polydeoxyribonucleotide chains of low molecular weight followed by elongation of these short chains by ligation to heavy DNA. This mechanism is quite analogous

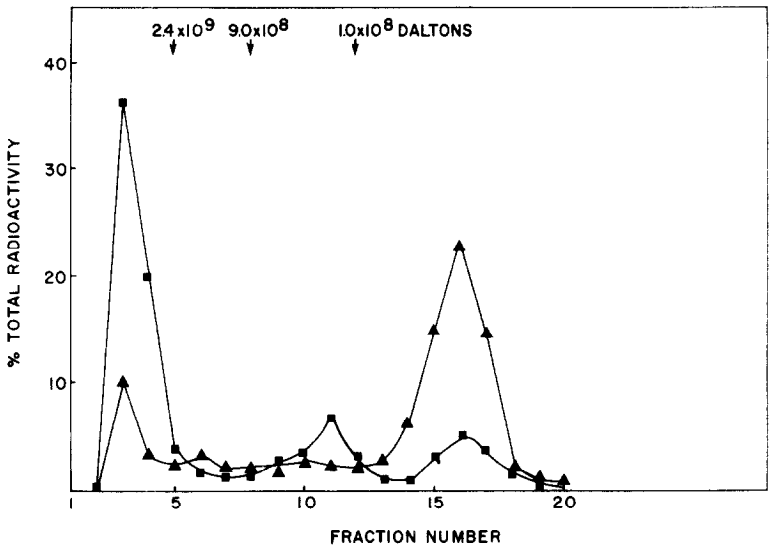

Fig. 2. Influence of ara-C on the kinetics of rat liver DNA chain elongation in vivo: Sedimentation of the newly made DNA in alkaline sucrose gradients. Rats were partially hepatectomized. 21 hr. later they were given 400 μc of tritiated thymidine intraperitoneally, and were killed 4 hr later. Ara-C (250 mg/kg) was given either at 5 (▲-▲-▲) or at 30 min (■-■-■), following the adiministration of thymidine-methyl-^{3}H. Total acid precipitable radioactivity recovered from each gradient was: 5 min pulse 654 cpm; 30 min pulse 957 cpm.

to that reported for bacterial cells and mammalian cells grown in tissue culture (12-14). Although ligases from mammalian cells have been isolated (15-17), the ligation step during replication in vivo has not yet been clearly demonstrated. In order to demonstrate the ligation step in vivo, the effect of ara-C on DNA chain elongation of prelabelled DNA was studied.

Results presented in Fig. 2 indicate that ara-C when administered at 5 min after the radioactive thymidine inhibited to a large extent the elongation of DNA during the next 3 hours and 55 min. However, the same agent had only a slight effect on the elongation process when given 30 min after the labeled thymidine.

At the concentration of ara-C used in these experiments, hepatic DNA synthesis was inhibited by 96% within 30 min. This percentage of inhibition persisted throughout the experimental period of 4 hours. In one experiment

thymidine-methyl-^{3}H (400 μC) was given 5 min after the administration of ara-C (250 mg/kg) and the rats were killed 4 hr. later. Sedimentation analysis in alkaline sucrose gradients of the newly made DNA indicated that all the acid-precipitable radioactivity is at the top of the gradient (less than 10^6 daltons) suggesting rapid cessation of chain elongation following ara-C administration.

Therefore it appears that in vivo hepatic DNA synthesis involves at least two steps, one which is sensitive to ara-C, possibly involving elongation by nucleotide addition upto an average size of 1×10^8 daltons and the second elongation by ligation which is insensitive to the effects of ara-C.

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