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EVIDENCE FOR INCREASED NUCLEOLAR RNA POLYMERASE ACTIVITY IN REGENERATING RAT LIVER

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ABSTRACT

Shu-Chen G. Chen (1977). *Evidence for Increased Nucleolar RNA Polymerase Activity in Regenerating Rat Liver.* Bull. Inst. Zool., Academia Sinica **16**(1): 63-67. The rate of nucleolar RNA synthesis increased more than three fold at 15 hours after partial hepatectomy. When the endogenous template function was blocked by actinomycin D and the native nucleolar RNA polymerase activity was measured with saturation levels of poly [d(A-T)] as template, the results indicate that the enhanced nucleolar RNA synthesis is largely due to the increased levels of RNA polymerase activity rather than to the derepression of the nucleolar genome.

After partial hepatectomy of the rat, a series of morphological and biochemical changes occur in the residual liver that result in the reconstitution of the original tissue mass[5]. Thus the regenerating liver system provides an excellent opportunity to study the biochemical events of gene activation during normal cell proliferation and rapid growth. Among the earliest detectable changes after partial hepatectomy is the increase in the rate of RNA synthesis with a concomitant increase in both the nucleolar size and the nucleolar RNA content[2,4,8,12].

Several reports in the literature have attempted to dissect the biochemical mechanism underlying the increased rate of RNA synthesis after hepatectomy. It could be due to the activation of RNA polymerase *per se* or the increased availability of DNA template for transcription, i. e. derepression of the genome. Tsukada and Liberman from a series of studies[14-16] using actinomycin D, puromycin and p-fluorophenylalanine injection to the partially hepatectomized rats at various time concluded that the increase in RNA polymerase activity after partial hepatectomy was a result of increased synthesis of RNA polymerase itself. On the other hand, Pogo *et al.*[10], Thaler *et al.*[13], and Mayfield *et al.*[7] evaluated the template activity of chromatin from regenerating liver using exogenous bacterial RNA polymerase and concluded that partial hepatectomy increased the template function. Because of these conflicting reports and the finding that the use of bacterial RNA polymerase to transcribe mammalian chromatin was an unreliable method to measure the true template function[3,6], this problem was

therefore reinvestigated in this study using the isolated nucleolar system and a technique which is able to distinguish whether the increased nucleolar RNA synthesis is due to an enhanced DNA template function or increased level of RNA polymerase activity *per se*.

MATERIALS AND METHODS

Partial Hepatectomy

Male Sprague-Dawley rats, weighing 200 to 300 g, were divided into two groups of 5 to 7 animals. Partial hepatectomy was performed according to Higgins and Anderson[5] under ether anesthesia, and approximately 70% of liver (median and left lateral lobes) was removed. The sham operation was performed in the same way except that no liver was removed.

Isolation of Rat-liver Nuclei and Nucleoli

The isolation procedure is essentially that of Muramatsu *et al.*[9]. The animals were sacrificed at the various time intervals after partial hepatectomy. The livers were excised after perfusion with cold 0.25 M sucrose-3.3 mM $CaCl_2$ solution. All subsequent operations were conducted at 0-4°C.

Livers from each group were homogenized in 10 volumes of 2.3 M sucrose 3.3-mM $CaCl_2$ in a glass Potter-Elrehjem homogenizer. The homogenate was filtered through cheese cloth and centrifuged at 40,000 xg for 1 hr. The nuclear pellet was then suspended in 0.34 M sucrose solution of the original volume of liver used. The suspension was sonicated with a Branson sonifier (Model No. S-110) until virtually all nuclei were broken. The sonicate was layered over 15 ml of 0.88 M sucrose and centrifuged at 2,000 xg for 20 min at 0°C. The pellet contained the highly purified nucleoli. The purity of the isolated nucleoli was verified by microscopic examination.

Assay for RNA Polymerase Activity in Rat-liver Nucleoli

RNA polymerase activity was measured *in vitro* with a reaction mixture containing in a final volume of 700 μl: 100 μl of nucleolar suspension (about 35 μg of DNA); 50 μmol Tris-HCl (pH 8.2); 14 μmol 2-mercaptoethanol; 2.5 μmol $MgCl_2$; 0.1 μmol each of cold ATP, UTP, CTP, and GTP; 2 μCi of (5-^{3}H)-UTP (22.2 Ci/mmol; New England Nuclear Corp.); various amount of poly [d(A-T)] (Miles Laboratories, Inc.); and 10 μg of actinomycin D (Nutritional Biochemicals, Inc.) as indicated in each experiment. The nucleoli were added last to initiate the reaction. All incubation flasks were run in triplicate, and were carried out at 35°C for 15 minutes with shaking. The reaction was terminated by the addition of 5 ml of cold 10% tricholoroacetic acid (TCA) containing 1% sodium pyrophosphate. The acid insoluble material was collected on Millipore filters (24 mm, type HA), which were then washed three times with 10 ml of 5% cold TCA containing 1% sodium pyrophosphate and once with 5 ml of 60% ethanol. The filters were air dried, added to 10 ml of Bray's solution and their radioactivities measured in a liquid scintillation counter. RNA polymerase activity was expressed as pmoles of (^{3}H)-UMP incorporated into RNA per mg of nucleolar DNA. DNA concentration was determined as described by Burton[1].

RESULTS AND DISCUSSION

RNA synthesis in the isolated nucleolar system was measured at the various time intervals after partial hepatectomy and compared with that of sham-operated control. The result is shown in Fig. 1. The rate of nucleolar RNA synthesis increased gradually after partial hepatectomy. The nucleoli incorporated (^{3}H)-UMP into RNA more than three-fold the rate of sham-operated control at 15 hours after operation. This is in good agreement with the results reported in the literature[10,12,15]. Therefore the following studies always used the regenerating liver at 15 hours after operation.

In order to differentiate the possibilities

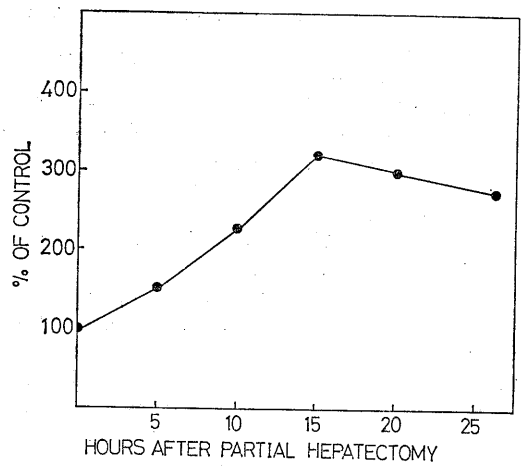

Fig. 1. Nucleolar RNA synthesis at the various time intervals after partial hepatectomy.

that the increased nucleolar RNA synthesis is due to increassed template availability of the nucleolar genome, or increassed amount and/or efficiency of RNA polymerase, or both, the following technique was employed. Because actinomycin D is a potent inhibitor of DNA-dependent RNA transcription by specific binding to deoxyguanosine moieties of DNA, the endogenous template function of nucleoli could be completely abolished by the addition of actinomycin D and the nucleolar RNA polymerase activity could be measured in the presence of synthetic template poly [d(A-T)], which is insensitive to actinomycin D inhibition due to the absence of deoxyguanosine moieties[11] and which codes for poly (U, A) synthesis. It was observed, as shown in Table 1, that a considerable portion of the increased ability to incorporate (^{3}H)-UMP into RNA by nucleoli isolated from regenerating liver was retained using poly [d(A-T)] as template. Thus it appears that the enhancement in nucleolar RNA synthesis in the regenerating liver mainly due to the increased level of RNA polymerase activity rather than the increased template function.

Uncertainty existed that the measured effect (see Table 1) varied depending upon the relative amount of poly [d(A-T)] added in the assay system. In order to further evaluate this parameter, various amount of poly [d(A-T)] were used to measure RNA polymerase activities of nucleoli isolated from the partially-hepatectomized and sham-operated animals. The results

TABLE 1

Comparison of RNA polymerase activity in the absence and presence of exogenous template in hepatic nucleoli derived from control and partial hepatectomized rats.

	Poly [d(A-T)] (μg)*	pmol of (^{3}H) UMP/mg DNA		% of Control
		Control liver	Regenerating liver	
(1)	0	1567±63	5183±176	330
	25	708±31	1917±89	270
(2)	0	1766±84	5939±251	336
	50	933±47	1817±106	195

* Actinomycin D (10 ug) was added only in the presence of poly [d(A-T)].

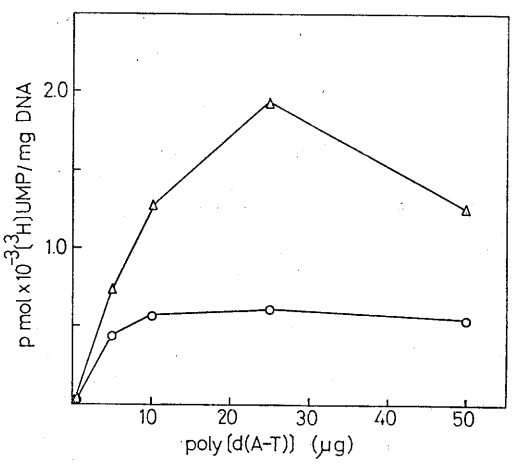

Fig. 2. The effect of varying concentration of exogenous template on hepatic nucleolar RNA polymerase activities of normal and regenerating livers. Actinomycin D (10 μg) was added in all the incubation mixture. Each point represents the average of three experiments.
○—○—○ Normal liver,
△—△—△ Regenerating liver.

from these series of studies are shown in Fig. 2. Apparently, at all concentrations of poly [d(A-T)] employed, the nucleolar RNA polymerase activity in the regenerating liver was always higher than that of sham-operated control. The reason why higher concentrations of poly [d(A-T)] (higher than 25 μg) were inhibitory is not clear. Nevertheless, when assayed at the optimal level of poly [d(A-T)] (25 μg), the nucleoli from regenerating liver exhibited almost three-fold increased nucleolar polymerase activity. Apparently, the present results indicate that the increased template function only plays a minor role in the enhanced RNA synthesis which occurs during hepatic regeneration. At present, whether this increased level of RNA polymerase activity is a result of increased amount of the enzyme or activation of the pre-existing enzyme is uncertain.

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再生鼠肝細胞核仁 RNA 合成酵素活性提高之證據

陳 淑 眞

老鼠肝臟切除三分之二，於十五小時以後，細胞核仁之 RNA 合成速率增加三倍以上。若用 Actinomycin D 阻斷細胞核內原有合成 RNA 模板之功能，並以飽和濃度之 Poly〔*d*(A-T)〕做爲模板，以測細胞核仁內 RNA 合成酵素之活性，實驗結果顯示增加之細胞核仁 RNA 生合成主要是由於細胞核仁 RNA 合成酵素活性的提高，而不是由於細胞核仁內基因之壓制去除。