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Studies on the Activity of α-Fetoprotein Gene

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The expression of the gene of α-fetoprotein (AFP) was studied in AH-66 hepatoma cells and adult rat liver cells.

With purified AFP mRNA as a template, [^{3}H]-labeled AFP complementary deoxyribonucleic acid (cDNA) (around 1000 nucleotides) was synthesized by the use of reverse transcriptase and was used for assaying the nucleotide sequence involved in AFP gene and in its transcripts by the hybridization technique.

Upon treatment with DNase I, the AFP gene in AH-66 nuclei was digested preferentially, whereas that in rat liver nuclei was not. These results show that the AFP gene exists in a relaxed conformation in AH-66 but takes a more condensed structure in rat liver. Micrococcal nuclease selectively cleaved the AFP gene in AH-66, as did DNase I. It also preferentially recognized the AFP gene in rat liver, although to a lesser extent than that in AH-66.

Chromosomal proteins extracted from AH-66 and rat liver chromatin were divided into four fractions with QAE-Sephadex A-25 ($CP_{0,0.1,0.25\text{ and }3}$ respectively). Liver chromatin was dissociated and reconstituted in the presence of each of AH-66 CP_{0-3} and, conversely, AH-66 chromatin was reconstituted in the presence of each of liver CP_{0-3}. Reconstituted chromatins were transcribed by *E.coli* RNA polymerase and the transcripts were annealed to AFP cDNA. AH-66 $CP_{0.1}$ activated the AFP gene transcription strongly, while none of the liver CP_{0-3} inhibited the expression of the gene.

Keywords——α-fetoprotein; AH-66 hepatoma cells; gene expression; chromosomal proteins; AFP cDNA; transcriptional control; nuclease; chromatin conformation; reconstituted chromatin

The fact that fetal liver and hepatoma cells produce α-fetoprotein (AFP), whereas adult liver cells do not synthesize it, can be interpreted in terms of the presence or absence of the expression of AFP gene, *i.e.*, the transcription of AFP gene to AFP messenger ribonucleic acid (mRNA) and the translation of the mRNA to AFP (so-called central dogma).

The deoxyribonucleic acid (DNA) content per cell is constant in various types of cell of a species, and it is supposed that each of the highly differentiated cells has substantially the same quality and quantity of hereditary information in the base sequences of DNA as those involved in the first fertilized egg. Each type of cell is expressing only the particular hereditary information essential for itself, which might be only a rather small percentage of all the genes contained. In other words, most of the genes in a given cell are inactive and their expression is being suppressed. It is considered, therefore, that many control mechanisms of gene expression are in action throughout the processes of transcription and translation. Among them, transcriptional control seems to be one of the more important and effective.

In eukaryotes, DNA is combined with histones, non-histone proteins and RNAs to constitute chromatin. The histones contribute to forming the nucleosome structure and condensing the chromatin, and are probably nonspecific suppressors for gene transcription. In addition, non-histone proteins are considered to participate in specific gene transcription.

To study the control mechanism involved in gene transcription, chromatin was digested by various nucleases,[1-3] since it was considered that the chromatin being actively transcribed would exist in a relaxed form which might be digested preferentially by the nuclease. Such a change in the chromatin structure would be a necessary but not sufficient condition for

transcription. Some other factors may be necessary for the transcription of the genes. *In vitro* transcription experiments using reconstituted chromatins were performed to identify such factors.[4-6] The results suggested that some species of non-histone proteins were required to cause the transcription of a particular gene.

The authors have been investigating the structure and the transcriptional control of AFP gene employing AH-66 ascites cells derived from rat hepatoma. This article reports the results so far obtained.

Materials and Methods

Synthesis of [^{3}H]cDNA to AFP mRNA——AFP mRNA was prepared as reported in the previous paper.[7] Tritiated cDNA to AFP mRNA was synthesized with RNA-dependent DNA polymerase of avian myeloblastosis virus (AMV) using the procedure described by Meyers *et al.*[8] The reaction mixture is as follows: 50 mM Tris-HCl (pH 8.3), 100 mM KCl, 6 mM $MgCl_2$, 10 mM dithiothreitol, 75 μg/ml of actinomycin D, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dTTP, 0.2 mM [^{3}H]-dCTP (22 Ci/mmol, Radiochemical Centre, Amersham), 25 μg/ml of Oligo(dT)$_{12-18}$ (P-L Biochemicals Inc.), 100 μg/ml of AFP mRNA, 125 units/ml of AMV reverse transcriptase (provided by Dr. Nishi of Hokkaido University). The mixture was incubated at 37°C 1 for h, followed by addition of SDS (0.5%), ethylenediaminetetraacetic acid (EDTA) (5 mM) and a mixture of phenol, chloroform and isoamyl alcohol (25: 25: 1). The whole was shaken for 3 min at room temperature, then the water phase was made up to 0.4 N NaOH and incubated at 37°C for 1 h. The mixture was neutralized with 4 N HCl and dialyzed against 10 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl and 10 mM EDTA. The specific radioactivity of AFP cDNA was approximately 1×10^7 cpm/μg.

Alkali-Sucrose Gradient Centrifugation of AFP cDNA——Analysis of AFP cDNA on an alkali–sucrose gradient was performed according to Sala–Trepat *et al.*[9] Poly(dA) (7.01-s) and poly(dC) (9.89-s) purchased from P-L Biochemicals Inc. were used as references.

RNA-cDNA Hybridization——Hybridization was performed by a modification of the procedure of Miura *et al.*[10] Each reaction mixture contained 10 mM Tris-HCl (pH 7.4), 0.3 M NaCl, 0.1% SDS, 1 mM EDTA, approximately 10000 cpm AFP cDNA and a suitable amount of RNA. The mixture was overlayed with paraffin oil to prevent evaporation. The mixture was heated at 100°C for 1 min and then incubated at 68°C. After incubation, aliquots were digested with 500 units/ml of S_1-nuclease (Boehringer Mannheim) in the presence of 0.3 M NaCl, 2.5 mM zinc acetate and 30 mM sodium acetate (pH 4.5) at 37°C for 1 h. The S_1-nuclease resistant hybrids were precipitated with 20% trichloroacetic acid (TCA) and collected on a Millipore filter (GS, 0.22 μm). The filter was dissolved in 10 ml of dioxane scintillation cocktail (naphthalene 60 g, DPO 4 g, POPOP 0.2 g, methanol 100 ml, ethylene glycol 20 ml and *p*-dioxane 850 ml), and the radioactivity was measured.

Thermal Denaturation of AFP cDNA: mRNA Hybrid——The thermal denaturation was determined according to Affara *et al.*[11] The cDNA: mRNA hybrids were heated at an appropriate temperature for 5 min and chilled rapidly. Aliquots were treated with S_1-nuclease as described above. The radioactivity of the hybrid resistant to S_1-nuclease digestion was measured.

Isolation of Nuclei——Nuclei were isolated from AH-66 cells and from liver cells as described by Stein *et al.*[12] Crude nuclear fractions were homogenized with 10 volumes of 2.2 M sucrose containing 10 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$ and 0.5 mM $CaCl_2$ and centrifuged at $80000 \times g$ for 1 h.

Nuclease Digestion of Nuclei and Gel-Filtration of Digested DNA——The nuclei were digested as described by Bloom *et al.*[13] Isolated nuclei were suspended in 0.35 M sucrose containing 10 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$ and 0.5 mM $CaCl_2$ (40 A_{260}/ml), and digested with DNase I (10 μg/ml, Boehringer Mannheim) at 0°C for 4 min or with micrococcal nuclease (75 units/ml, P-L Biochemicals Inc.) at 37°C for 1.5 min. The mixtures were then made up to 1 M NaCl and 1% SDS, and deproteinized by two extractions with chloroform/isoamyl alcohol (24: 1). The water phase was made up to 0.4 N NaOH and incubated at 37°C for 2 h to hydrolyze RNA. Samples were neutralized with 4 N HCl and extracted with chloroform. DNA was precipitated with ethanol and fractionated by gel–filtration on Sepharose-4B equilibrated with 5 mM Tris-HCl (pH 8) containing 50 mM NaCl and 10 mM EDTA.

Electrophoresis of DNA——DNA samples were analyzed on 3% polyacrylamide slab gels containing 40 mM Tris-HCl (pH 7.8), 20 mM sodium acetate and 2 mM EDTA.[14]

DNA-cDNA Hybridization——DNA-cDNA hybridization was performed by a modification of the procedure described by Bloom *et al.*[15] A half ml of each reaction mixture consisted of 200 μg of DNA to be assayed and 20000 cpm of AFP cDNA in 20 mM Tris-HCl (pH 7), 0.6 M NaCl and 3 mM EDTA. Samples were heat–denatured and immediately placed at 68°C. The extent of hybrid formation was determined by measurement of the resistance to S_1-nuclease digestion, as described above.

Preparation and Fractionation of Chromosomal Proteins——Chromatin was prepared according to Garrard *et al.*[16] Preparation and fractionation of chromosomal proteins were performed according to Park *et al.*[4] The chromatin was homogenized with 10 mM Tris-HCl (pH 8.3) containing 5 M urea (Solution A) + 3 M

NaCl (0.5 mg/DNA ml), and the homogenate was stirred for 4 h at 0°C. The homogenate was centrifuged at 150000×*g* for 36 h to precipitate DNA. The supernatant containing chromosomal proteins was dialyzed at 4°C against 4 changes of 10 volumes of Solution A and loaded on a column of QAE-Sephadex A-25 at a flow rate of 15—30 ml/cm²/h, using approximately 1 g of Sephadex per 10 mg of protein. The column was then eluted with 2.5 column volumes each of Solution A containing 0, 0.1, 0.25 or 3 M NaCl. The eluted fractions were named CP_0, $CP_{0.1}$, $CP_{0.25}$ and CP_3, respectively.

Reconstitution of Chromatin——Reconstitution of chromatin was carried out by the following procedure, as described by Gould *et al.*[17] An equivalent amount of one of AH-66 CP_{0-3} was added to rat liver chromatin dissociated in Solution A+3 M NaCl, or alternatively, an equivalent amount of one of liver CP_{0-3} was added to AH-66 chromatin. For gradual reconstitution of the chromatin, each mixture was successively dialyzed for 2 h each against 10 volumes of 10 mM Tris-HCl (pH 8.3) containing 2, 1, 0.6, 0.4, 0.2 and 0 M NaCl. The reconstituted chromatin was recovered by centrifugation at 30000×*g* for 1 h.

Transcription of Chromatin——Chromatin was transcribed using *E.coli* RNA polymerase according to the procedure of Gadski *et al.*[18] The composition of the reaction mixture is as follows: 50 mM Tris-HCl (pH 7.9), 150 mM NaCl, 1 mM $MnCl_2$, 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM NTP, 100 μg DNA/chromatin ml, 10 units/ml of *E.coli* RNA polymelase (Boehringer Mannheim). After incubation at 37°C for 1 h, the mixture was centrifuged at 12000×*g* for 20 min. The supernatant was treated with 50 μg of DNase I (Boehringer Mannheim) at 37°C for 30 min, and subsequently was brought to a concentration of 0.5% SDS and 20 mM EDTA. After addition of 1 volume of a mixture of phenol, chloroform and isoamyl alcohol (25 : 25 : 1), the mixture was shaken for 3 min at room temperature, and centrifuged at 1000×*g* for 10 min. Two volumes of ethanol was added to the water phase, and the mixture was left to stand overnight at −20°C. RNA was then precipitated by centrifugation at 10000×*g* for 30 min.

Results

Properties of AFP cDNA

The distribution of molecular weight of the synthesized [³H]cDNA was analyzed by alkali–sucrose gradient centrifugation (Fig. 1). The largest cDNA was found to be of about 1600 nucleotides length, but the peak fraction was of about 1000 nucleotides length, which corresponded to 37% of the size of AFP mRNA. This peak fraction was used for the hybridization experiments described below.

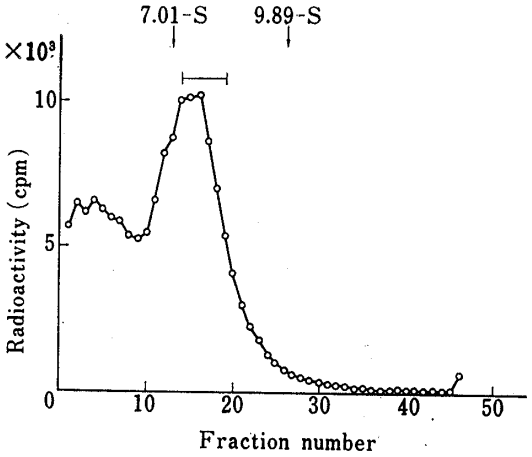

Fig. 1. Analysis of AFP cDNA by Alkali–Sucrose Gradient Centrifugation

Tritiated AFP cDNA was centrifuged at 87000× *g* for 66 h through a 15 to 30% sucrose gradient in 0.1 N NaOH and 0.9% NaCl. Fractions of 0.3 ml each were collected and the radioactivity in a 10 μl aliquot of each fraction was measured with 1 ml of water and 10 ml of toluene–Triton scintillation cocktail. Arrows indicate the positions of DNA markers. The fraction indicated by a horizontal bar was used for hybridization experiments.

Fig. 2 shows the result of a hybridization experiment between AFP cDNA and AFP mRNA. AFP cDNA hybridized with AFP mRNA with a $Crot_{1/2}$ value of 2×10^{-3} (moles of ribonucleotides×s/l). On the other hand, AFP cDNA did not hybridize with rat liver catalase mRNA (provided by Dr. Sakamoto of our laboratory) at all.

The fidelity of AFP cDNA was investigated by analyzing the thermal denaturation of AFP cDNA: mRNA hybrids formed at high Crot value (Fig. 3). A sharp melting profile was obtained with a Tm of 88°C, indicating that a faithful transcript of considerable size had been obtained.

Sensitivity to Nucleases of AFP Gene

Nuclei of AH-66 hepatoma cells and of rat liver cells were digested with DNase I or micrococcal nuclease to study the conformation of the AFP gene. Bloom *et al.*[13] reported that DNase I could digest the DNA binding to the nucleosome core whereas micrococcal nuclease could digest only the DNA in the linker region.

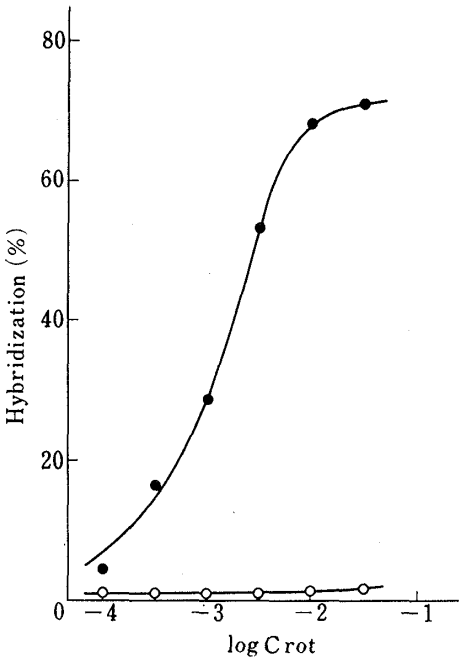

Fig. 2. Hybridization of AFP cDNA with AFP mRNA

AFP cDNA was incubated with AFP mRNA (●) or rat liver catalase mRNA (○). S_1-nuclease resistant radioactivity was assayed as described in Materials and Methods. Crot: moles of ribonucleotides × seconds/liter.

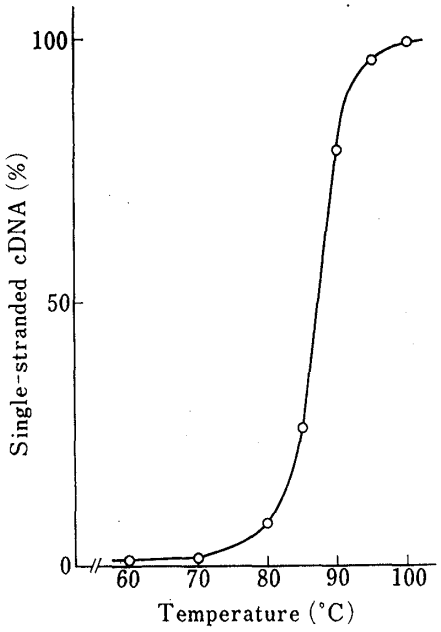

Fig. 3. Thermal Denaturation of Hybrids formed between AFP cDNA and AFP mRNA

Hybrids were heated at an indicated temperature for 5 min, chilled rapidly and treated with S_1-nuclease. The amount of hybrids surviving S_1-nuclease digestion was plotted against temperature.

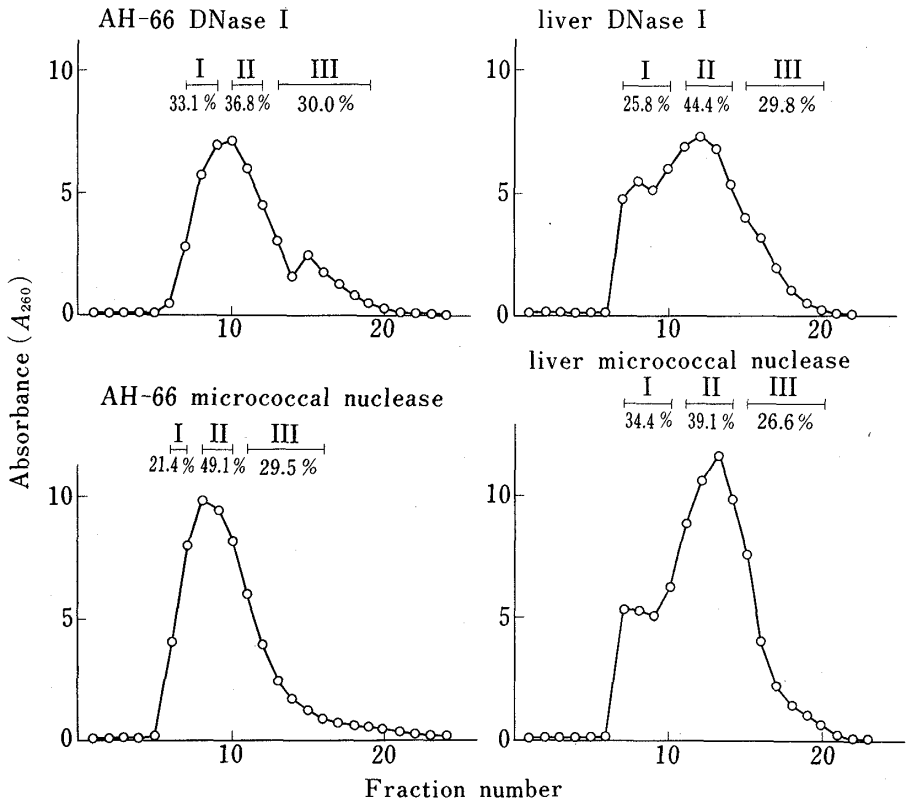

Fig. 4. Gel-Filtration of Nuclease–Digested DNA with Sepharose–4B

Approximately 20 mg of nuclease-digested DNA was gel-filtrated on a Sepharose-4B column (2.2 × 35 cm) at a flow rate of 5—10 ml/h. Eluted DNA was divided into three fractions (I, II, III).

1) **Gel-filtration of Nuclease-digested DNA**——DNA was extracted from nuclease-digested nuclei and gel-filtrated with Sepharose-4B (Fig. 4). Digested DNA was divided into three fractions as shown by horizontal bars. Each fraction contained the indicated percentage of DNA.

Fractionated DNA was analyzed by electrophoresis on 3% polyacrylamide gels. Undigested total DNA contained only small amounts of low molecular weight DNAs, but larger amounts were present in the digested DNA. As was expected, fraction III consisted of the smaller DNA, II of intermediate size DNA and I of the larger DNA.

2) **Hybridization of Various DNA Fractions with AFP cDNA**——At first, AFP cDNA was hybridized with the undigested DNA from AH-66 and from rat liver nuclei (Fig. 5). Both DNAs gave similar values of hybridization percentage. This result indicates that a rat liver cell contains the same quantity of AFP gene as an AH-66 hepatoma cell.

Next AFP cDNA was hybridized with the DNase I-digested DNA (Fig. 6). In AH-66, fraction III showed the highest hybridization and fraction I the lowest. From these results it is considered that the AFP gene in AH-66 nuclei exists in a relaxed structure which is digested by DNase I preferentially. However, in the case of rat liver, the three DNA fractions showed no difference in hybridization with AFP cDNA, suggesting that the AFP gene exists in a rather condensed structure.

In the third place, AFP cDNA was hybridized with the micrococcal nuclease-digested DNA (Fig. 7). In AH-66, fraction III showed the highest hybridization, as was the case in DNase I digestion. The AFP gene in rat liver was susceptible to micrococcal nuclease (in contrast to DNase I). However, the extent of digestion seems to be slightly less in rat liver than in AH-66.

Effect of Chromosomal Proteins on Transcription of AFP Gene

1) **Fractionation of Chromosomal Proteins**——Chromosomal proteins extracted from AH-66 and rat liver chromatin with 5 M urea and 3 M NaCl were divided into four fractions

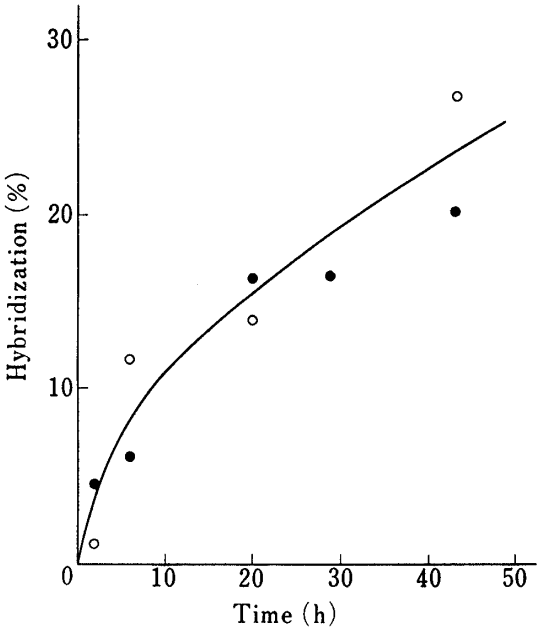

Fig. 5. Hybridization with AFP cDNA of Undigested DNA

DNA extracted from AH-66 (●) and rat liver (○) nuclei was annealed to AFP cDNA for the indicated times. S_1-nuclease resistant radioactivity was measured as described in "Materials and Methods."

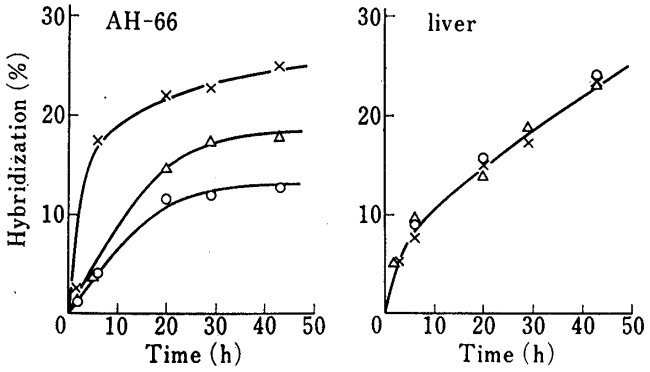

Fig. 6. Hybridization with AFP cDNA of Fractionated DNAs from DNase I-Digested Nuclei

DNA was extracted from DNase I-digested nuclei and fractionated with Sepharose-4B. Fractionated DNAs were annealed to AFP cDNA for the indicated times. S_1-nuclease resistant radioactivity was measured as described in "Materials and Methods." ○: DNA fraction I, △: DNA fraction II, × :DNA fraction III.

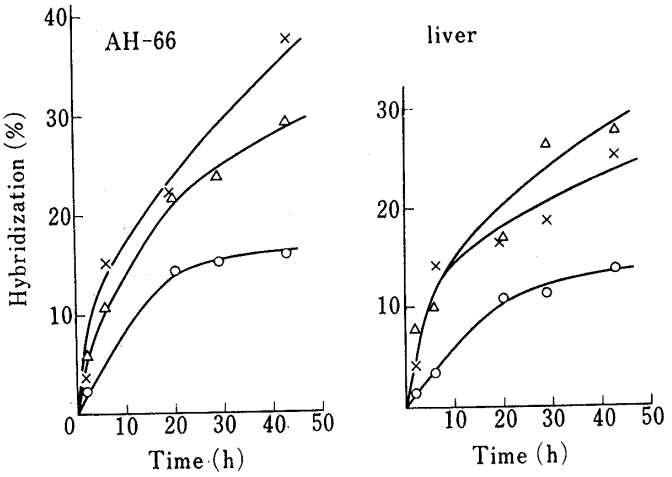

Fig. 7. Hybridization with AFP cDNA of Fractionated DNA from Micrococcal Nuclease–Digested Nuclei

DNA was extracted from micrococcal nuclease-digested nuclei and fractionated with Sepharose-4B. Fractionated DNAs were annealed to AFP cDNA for the indicated times. S_1-nuclease resistant radioactivity was measured as described in Materials and Methods. ○: DNA fraction I, △: DNA fraction II, × :DNA fraction III.

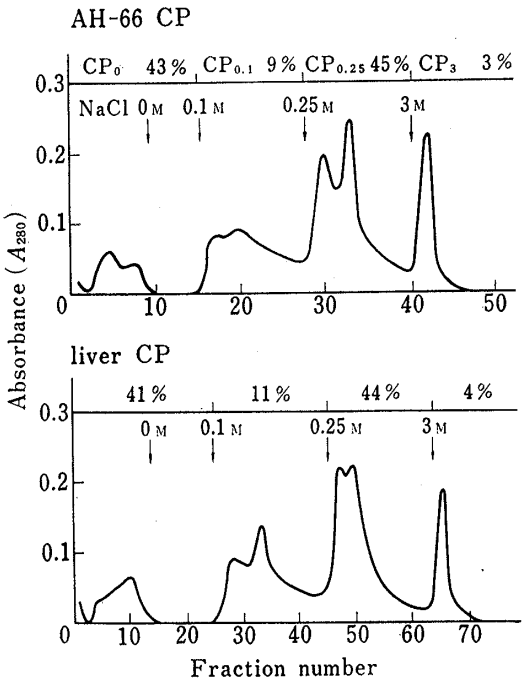

Fig. 8. Elution Profile of Chromosomal Proteins from QAE–Sephadex A-25

Chromosomal proteins were loaded onto a QAE-Sephadex A-25 column, and eluted with Tris-HCl buffer(pH 8.3)containing urea and increasing amounts of NaCl (0.1, 0.25, 3 M) successively. For details, see Materials and Methods. The amount of proteins in each fraction(percentage)is shown in the upper panel.

($CP_{0,0.1,0.25,3}$) by chromatography on QAE-Sephadex A-25 (Fig. 8). Each CP fraction contained the amount of proteins (percent) indicated in the figure. No significant difference was detectable in the elution profile of CP between AH-66 and rat liver.

Each CP fraction was analyzed by SDS-PAG electrophoresis. It was found that CP_0 passing through the QAE-Sephadex A-25 column contained only histones, whereas $CP_{0.1,0.25,3}$ contained many non-histone proteins both in AH-66 and in rat liver.

2) **Hybridization of *in Vitro* Transcripts from Various Chromatin Preparations with AFP cDNA**——AFP cDNA was hybridized with RNAs transcribed from native and reconstituted chromatins of AH-66 cells and rat liver by *E.coli* RNA polymerase (Fig. 9). The figure shows that the transcripts from intact and reconstituted chromatins of AH-66 cells hybridized with AFP cDNA, but the transcripts from those of rat liver cells did not. The results demonstrate that the AFP gene is actively expressed in AH-66 cells, but effectively suppressed in liver cells. The results also suggest that the reconstitution procedure did not cause a functional change of the AFP gene.

Transcripts from liver chromatin reconstituted in the presence of each fraction of AH-66 CP_{0-3} were annealed to AFP cDNA (Fig. 10). The addition of AH-66 $CP_{0.1}$ during the reconstitution of rat liver chromatin leads to a remarkable transcription of AFP gene, which is otherwise inactive. AH-66 $CP_{0.25}$ also showed a little activity for transcribing liver AFP gene, but neither CP_0 nor CP_3 had activity.

Fig. 11 shows the results of a reverse experiment. AH-66 chromatin was reconstituted in the presence of each of liver CP_{0-3}, and transcribed, then the transcripts were annealed to AFP cDNA. None of the liver CP fractions inhibited the transcription of AH-66 AFP gene.

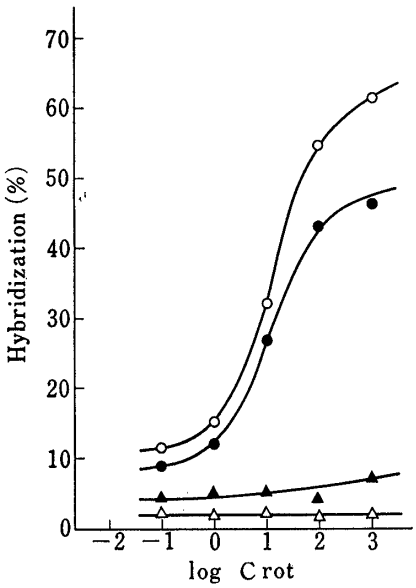

Fig. 9. Hybridization with AFP cDNA of RNAs transcribed from Intact and Reconstituted Chromatins

AFP cDNA was incubated with RNAs transcribed from intact AH-66 chromatin (○), reconstituted AH-66 chromatin (●), intact liver chromatin (△) and reconstituted liver chromatin (▲). S_1-nuclease resistant radioactivity was measured as described in Materials and Methods.

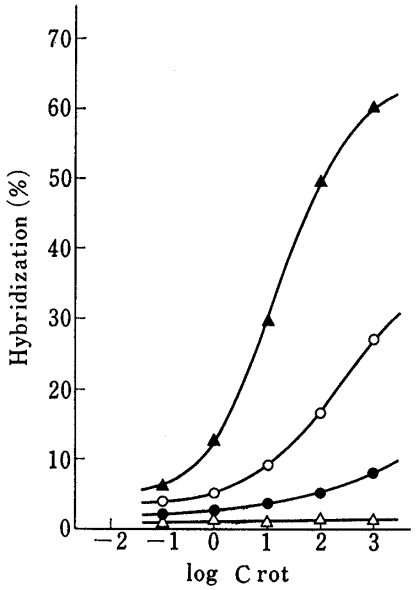

Fig. 10. Hybridization with AFP cDNA of RNAs transcribed from Liver Chromatin reconstituted in the Presence of AH-66 CP_{0-3}

AFP cDNA was incubated with RNAs transcribed from liver chromatin reconstituted in the presence of AH-66 CP_0 (△), $CP_{0.1}$ (▲), $CP_{0.25}$ (○) or CP_3 (●). S_1-nuclease resistant radioactivity was measured as described in Materials and Methods.

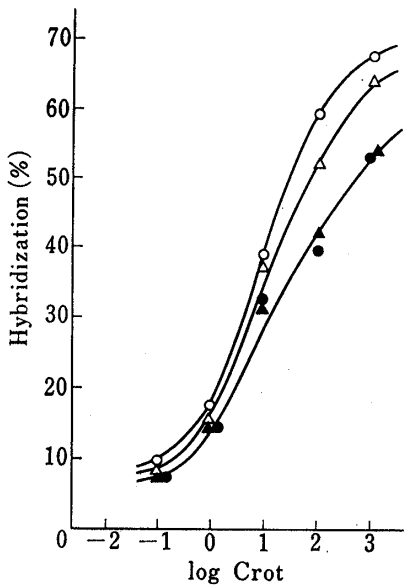

Fig. 11. Hybridization with AFP cDNA of RNAs transcribed from AH-66 Chromatin reconstituted in the Presence of Rat Liver CP_{0-3}

AFP cDNA was incubated with RNAs transcribed from AH-66 chromatin reconstituted in the presence of rat liver CP_0 (△), $CP_{0.1}$ (▲), $CP_{0.25}$ (○) or CP_3 (●). S_1-nuclease resistant radioactivity was measured as described in Materials and Methods.

Discussion

Weintraub and Groudine[1] reported that globin genes in red blood cell nuclei were digested selectively by DNase I, but those in fibroblasts and in brain cells were not. They claimed that active genes were likely to be packed by histones of an altered conformation that rendered the associated DNA extremely sensitive to DNase I digestion. Garel and Axel[2] also described a selective cleavage of transcriptionally active ovalbumin gene by DNase I. The gene in hen oviduct was preferentially digested, but the one in liver was not. Those authors obtained negative results with the ovalbumin gene in chick embryo red blood cells[1] and the globin gene in hen oviduct,[19] which were transcriptionally inactive. In the present study, AFP genes in AH-66 cells (actively engaged in AFP mRNA synthesis) were digested preferentially by DNase I, whereas the same genes in adult rat liver cells (transcriptionally silent) were not sensitive to the digestion. Therefore, the AFP genes are likely to exist in a relaxed conformation in the former cells, but to form a rather condensed structure in the latter cells.

Recently, Bloom and Anderson[13] obtained evidence that the globin genes in both immature and mature erythrocyte nuclei were destroyed by DNase I much more rapidly than the greater part of the nuclear DNA, and they suggested that the DNase I-sensitive structure remains asociated with globin sequences following their inactivation. According to their report,[13] micrococcal nuclease, instead of DNase I, cleaved preferentially the expressed globin genes in immature erythrocyte nuclei, but not the inactive globin genes in mature erythrocyte nuclei. However, the earlier paper by Weintraub and Groudine[1] reported the digestion by DNase I of two types of globin genes in different red cell lines, *i.e.*, embryonic and adult red cells, suggesting that embryonic but not adult-specific globin sequences were digested in embryonic cells and *vice versa*.

In contrast to the findings by Bloom and Anderson[13,15] and Bellard *et al.*,[20] micrococcal nuclease did not preferentially recognize the transcriptionally active AFP genes in the present study. The AFP genes in adult rat liver cells, in which AFP mRNA synthesis was no longer observed, were also cleaved by micrococcal nuclease although to lesser extent than in the case of those in AH-66 cells. Similar results were reported with the globin genes[1] and the ovalbumin genes.[2] The discrepancy may be partly due to the extent of digestion, or the suggestion by Bloom and Anderson[13] that the micrococcal nuclease–sensitive conformation of the ovalbumin and globin genes in chromatin is dynamically related to the expression of these sequences may not be generally applicable to other genes.

It seems to be of much interest that $CP_{0.1}$ of AH-66 nuclei led to the transcription of AFP gene of liver chromatin. Some proteins in the AH-66 $CP_{0.1}$ fraction are considered to have changed the inactive AFP gene in liver chromatin to its active form, or (and) activated a promoter of liver AFP gene. On the other hand, none of the liver CP_{0-3} inhibited the transcription of AFP gene of AH-66 chromatin. These experimental results can tentatively be explained in the following two ways. The first is that the liver CP does not contain any factor activating the AFP gene, whereas AH-66 $CP_{0.1}$ does. The alternative is that the liver CP contains an inhibitor for the transcription of AFP gene whereas the AH-66 CP contains an activator, which binds to the gene preferentially during the reconstitution process. It is thought that histone I and non-histone chromosomal proteins (NHCP) are involved in the change in conformation of chromatin, and NHCP also participate in the control of gene expression. There are many varieties of NHCP in chromatin, and no specific one concerned with the conformational change or the transcriptional control of a particular gene has yet been isolated. It would be interesting to attempt to isolate such a specific NHCP and to investigate its functions.

Recently, discussions have appeared on *in vitro* experiments for the transcription of reconstituted chromatins. One problem is concerned with the reconstruction procedure. The procedures employing urea and NaCl are most widely used at present, but such conditions are not physiological, so that there is a danger of failing to reconstitute the original structure of chromatin. Among the procedures using urea and NaCl, the one by Gould *et al.*,[17] employed in the present study, was reported to reproduce the nucleosome structure most faithfully.[21]

Another discussion centers around the species of RNA polymerase to be used for transcribing the chromatin. RNA polymerase of *E.coli* is most common since it is rather difficult to prepare the enzyme of eukaryotic origin in large quantities. It is unclear whether or not prokaryotic RNA polymerase can faithfully transcribe the eukaryotic genome. It was reported that larger amounts of RNA were transcribed from eukaryotic chromatin by the homologous RNA polymerase than when a prokaryotic enzyme was used.[22,23] Mammalian RNA polymerase II transcribed the globin gene in bone marrow chromatin three times as efficiently as the *E.coli* enzyme.[24] It may be necessary to study the reconstitution of chromatin under more physiological conditions and to carry out the transcription as faithfully as *in vivo* by using a homologous RNA polymerase.

Although the relationship between AFP and carcinogenesis is still unknown, it is an interesting possibility that the growth of cancer cells producing AFP may be inhibited when the expression of the AFP gene is suppressed. Furthermore, AFP is undoubtedly an excellent material for studies of the regulation of gene transcription. Work is in progress to determine whether or not AH-66 $CP_{0.1}$ changes the AFP gene structure of adult liver, and the authors are also trying to isolate from AH-66 CP the factor(s) which renders the AFP gene transcriptionally active.

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References and Notes

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