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Role of Endogenous Regucalcin in Nuclear Regulation of Regenerating Rat Liver: Suppression of the Enhanced Ribonucleic Acid Synthesis Activity

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Abstract The role of endogenous regucalcin in the regulation of ribonucleic acid (RNA) synthesis activity in the nucleus of normal and regenerating rat livers was investigated. Nuclear RNA synthesis was measured by the incorporation of [^{3}H]-uridine 5′-triphosphate into the nuclear RNA in vitro. The presence of regucalcin (0.25 or 0.5 μM) in the reaction mixture caused a significant decrease in nuclear RNA synthesis of normal rat liver. α-Amanitin (10^{-8}–10^{-6} M), an inhibitor of RNA polymerase II and III, decreased significantly nuclear RNA synthesis activity. The effect of regucalcin (0.25 μM) in decreasing nuclear RNA synthesis activity was not seen in the presence of α-amanitin (10^{-6} M). The calcium chloride (10 μM)-increased nuclear RNA synthesis activity was significantly suppressed by the addition of regucalcin (0.25 μM). RNA synthesis activity was significantly enhanced in the nuclei of regenating rat liver obtained at 24, 48, or 72 h after partial hepatectomy. This enhancement was significantly inhibited in the presence of PD98059 (10^{-5} M), staurosporine (10^{-6} M), or vanadate (10^{-3} M). Western analysis of the nuclei of regenerating liver obtained at 24, 48, or 72 h after partial hepatectomy showed a significant increase in regucalcin protein as compared with that of sham-operated rats. The presence of anti-regucalcin monoclonal antibody (25 or 50 ng/ml) in the reaction mixture caused a significant increase in nuclear RNA synthesis activity of normal rat liver. This increase was completely blocked by the addition of regucalcin (1.0 μM). The effect of anti-regucalcin monoclonal antibody (50 ng/ml) in increasing nuclear RNA synthesis activity was significantly enhanced in the nuclei of regenerating liver obtained at 24, 48, or 72 h after partial hepatectomy. This enhancement was significantly suppressed by the addition of α-amanitin (10^{-6} M), PD98059 (10^{-5} M), staurosporine (10^{-6} M), or vanadate (10^{-3} M) in the reaction mixture. The present study demonstrates that endogenous regucalcin has a suppressive effect on the enhancement of RNA synthesis activity in the nucleus of regenerating rat liver with proliferative cells. J. Cell. Biochem. 87: 450–457, 2002.

Key words: regucalcin; RNA synthesis; cell proliferation; nucleus; regenerating rat liver

Calcium ion (Ca^{2+}) plays a pivotal role in the regulation of many cell functions. Liver metabolism is regulated by Ca^{2+} which is increased in the cytoplasm of liver cells by hormonal stimulation [Williamson et al., 1981; Kraus-Friedman and Feng, 1996]. Ca^{2+} has been demonstrated to play a role in liver nuclear function [Backs and Carafoli, 1987; Jones et al., 1989; Allbritton et al., 1994; Martelli et al., 1999]. The existence of an ATP-stimulated Ca^{2+} sequestration system in rat liver nuclei that generates a net increase in nuclear matrix free Ca^{2+} concentration has been demonstrated [Nicotera et al., 1989; Yamaguchi and Oishi, 1993]. Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C, which can amplify the effect of Ca^{2+}, exist in liver nucleus [Bosser et al., 1993; Matter et al., 1993]. Deoxyribonucleic acid (DNA) and RNA syntheses in liver nucleus may be partly mediated through Ca^{2+}-dependent protein kinases [Pardo and Fernandez, 1982; Backs et al., 1990].

Regucalcin plays an important role as a regulatory protein of Ca^{2+} signaling in many cells [Yamaguchi, 2000a,b]. Regucalcin has been demonstrated to translocate into rat liver nucleus [Omura and Yamaguchi, 1999a; Tsurusaki

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et al., 2000]. Regucalcin can inhibit Ca^{2+}/calmudulin-dependent protein kinase, protein kinase C, and Ca^{2+}-dependent protein phosphatase activities in rat liver nucleus [Katsumata and Yamaguchi, 1998; Omura and Yamaguchi, 1999b]. Moreover, regucalcin has been shown to suppress DNA synthesis activity in rat liver nucleus [Yamaguchi and Kanayama, 1996; Tsurusaki and Yamaguchi, 2002]. Thus regucalcin regulates liver nuclear function.

Regenerating rat liver stimulates proliferation of liver cells. Regucalcin has been demonstrated to suppress the enhancement of Ca^{2+}-dependent protein kinase [Katsumata and Yamaguchi, 1998], protein phosphatase [Omura and Yamaguchi, 1999b], and DNA synthesis activity [Tsurusaki and Yamaguchi, 2002] in the nucleus of regenerating rat liver, suggesting that regucalcin has a suppressive effect of the overexpression of liver cell proliferation.

Regucalcin has been shown to inhibit RNA synthesis in rat liver nucleus [Yamaguchi and Ueoka, 1997]. Whether regucalcin has a regulatory effect on RNA synthesis in the nucleus of regenerating rat liver with proliferative cells, however, has not been fully clarified. The present study was undertaken to determine the role of endogenous regucalcin in the regulation of nuclear RNA synthesis in regenerating rat liver after partial hepatectomy. We found that endogenous regucalcin levels are increased in the nucleus of regenerating rat liver, and that the protein plays a suppressive role in the enhancement of nuclear RNA synthesis in proliferative liver cells.

MATERIALS AND METHODS

Chemicals

[Methyl-^{3}H] uridine 5′-triphosphate ([5,6-^{3}H]-UTP; 1.85 TBq/mmol) was obtained from New England Nuclear (Boston, MA). α-Amanitin, PD98059 and staurosporine were purchased from Sigma Chemical Co. (St Louis, MO). Calcium chloride, vanadate, and other reagents were obtained from Wako Pure Chemical Co. (Osaka, Japan). The regents were dissolved in distilled water or 50% ethanol solution. Some reagents were passed through ion-exchange resin to remove metal ions.

Animals

Male Wistar rats (80–100 g, Japan SLC, Inc., Hamamatsu, Japan) were fed commercial laboratory chow (solid, Oriental Yeast Co., Ltd., Tokyo) containing 57.5% carbohydrate, 1.1% calcium, and 1.1% phosphorus, and distilled water, ad libitum.

Isolation of Regucalcin

Regucalcin is markedly expressed in rat liver cytosol [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993] from which it was isolated. Regucalcin in the cytosol fraction (the supernatant of 105,000*g*) of rat liver homogenate was purified to electrophoretic homogeneity by gel filtration on Sephadex G-75 and G-50 followed by ion-exchange chromatography on diethylaminoethyl (DEAE)-cellulose, as reported previously [Yamaguchi and Yamamoto, 1978].

Anti-Regucalcin Antibody

Anti-regucalcin antibody was a monoclonal antibody raised against regucalcin prepared using standard methods [Omura and Yamaguchi, 1999c]. Mouse (BALB/C, Japan SLC) were subcutaneously injected with 0.1 mg/animal of antigen (rat liver RC) emulsified with Freund's complete adjuvant, and 19 days later antigen (0.25 mg/animal) was intraperitoneally injected with Freund's incomplete adjuvant. Animals were sacrificed by bleeding 3 days after the last injection. Spleen cells were prepared from immuned mouse and fused into myeloma cells. Anti-regucalcin monoclonal antibody-producing cells (hybridoma cells) were obtained by screening. The IgG from hybridoma cells was isolated through a protein A-agarose column (Sigma).

Hepatectomy

Partial hepatectomy was carried out by excision of two-third of the liver (the medium and left laternal lobes), as described by Higgins and Anderson [1931]. Rats were anesthesized with either before surgery. In sham-operated rats, an incision was made, and the liver was manipulated, but not ligated. Surgery was routinely performed between 10:00 and 12:00 h.

Isolation of Nuclei

The nuclei from liver were isolated by the procedure of Jones et al. [1989] with a minor modification. Liver was homogenized in a Potter–Elvehjem homogenizer with a Teflon pestle in 40 ml of TKM solution (50 mM Tris-HCl, pH 4.5, 25 mM KCl, 5 mM $MgCl_2$) containing 0.25 M sucrose and 1.0 mM EGTA.

The homogenate was filtered through three layers of cheesecloth. The homogenate was centrifugated at 700*g* for 10 min. The pellets were homogenized (five strokes) in 40 ml of the same solution and centrifuged again at 700*g* for 10 min. The pellet was resuspended in 24 ml of the same solution by homogenization (five strokes), and 6 ml was added to each of four tubes containing 12 ml of TKM including 2.3 M sucrose. The upper layer and the sucrose cushion were removed with an aspirator. Then it was resuspended in ice-cold 0.25 M sucrose solution by hand homogenization to assay RNA synthesis and to analysis Western blotting for regucalcin. Assay of marker enzymes showed that there was less than 5% contamination by microsomes, plasma membranes, or mitochondria. DNA content in the nuclei was determined using the diphenylamine reaction [Burton, 1956]. Protein concentration was determined by the method of Lowry et al. [1951].

Estimation of RNA Synthesis

RNA synthesis by isolated liver nuclei was estimated by the procedure of Weiss [1960] with a minor modification. RNA synthesis was measured for 15 min at 37°C in mixture (1.0 ml) that contained 100 mM Tris-HCl buffer (pH 8.05), 5 mM $MgCl_2$, 10 mM cystein, 0.06 mM ATP, CTP, GTP, UTP ([^{3}H]UTP), and the suspension of intact liver nuclei (0.1 ml containing 30–50 μg DNA; 300–500 μg protein). Regucalcin (0.1–0.5 μM) or anti-regucalcin monoclonal antibody (10–50 ng/ml) was added as indicated. After incubation for 15 min at 37°C, a 100 μl sample of the reaction mixture was immediately filtered through a 0.22 μm pre-wetted Whatman filter (CF/C). The precipitate was washed with ice-cold trichloroacetic acid (5% TCA; 5 ml), and then washed with ethanol–ether (3:1). The filter were transferred to a scintillation vial and counted for radioactivity. The radioactivity in nuclear RNA was measured in a hyaminetoluene liquid scintillation mixture, and all the data were corrected for the incorporation with control mixture that lacked the three unlabeled nucleotides. RNA synthesis was expressed as distintegrations per minute (dpm) per milligram of nuclear DNA.

Western Blot Analysis

The nuclei from liver homogenate were used for Western blot analysis [Wessendorf et al., 1993; Tsurusaki et al., 2000]. Aliquots of nucleus containing 50 μg of protein were mixed with 5 × sample buffer, boiled for 5 min, and SDS–PAGE was performed by the method of Laemmli [1970] using 12% polyacrylamide resolving gel. After SDS–PAGE, the proteins were then transferred onto a polyvinylidene difluoride membrane at 100 mA for 4 h. The membranes were incubated with a polyclonal rabbit anti-regucalcin antibody [Yamaguchi and Isogai, 1993], which was diluted 1:2,000 in 10 mM Tris-HCl, pH 8, containing 150 mM NaCl, 0.1% (w/v) skim milk for 1 h. The membranes incubated with antibody were washed four times with washing buffer. Then membranes were incubated for 1 h with horseradish peroxidase linked anti-rabbit IgG, which was diluted 1:5,000 with washing buffer containing 5% (w/v) skim milk, and washed again. Detection of the protein bands was performed using an enhanced chemiluminescent kit following the manufacture's instructions. The molecular size of the detecting protein was determined by running the standard protein molecules of known sizes in parallel. The density of protein bands was quantified by densitometer scanning (Dual wavelength Flying-spot Scanner, CS-9000, Shimadzu Company, Japan).

Statistical Analysis

Data were expressed as the mean ± SEM. The significance of the difference between the values was estimated by Student's *t*-test. We also used a multiway ANOVA comparison test to compare the treatment groups. A P-value < 0.05 was considered to indicate a statistically significant difference.

RESULTS

Effect of Regucalcin on Nuclear RNA Synthesis Activity in Normal Rat Liver

The effect of regucalcin addition on RNA synthesis activity in the nuclei isolated from normal rat liver is shown in Figure 1. The reaction mixture contained either vehicle or regucalcin (0.1, 0.25, or 0.5 μM). Nuclear RNA synthesis activity was significantly decreased in the presence of regucalcin (0.25 or 0.5 μM). α-Amanitin is known as an inhibitor of RNA polymerase II and III. The presence of α-amanitin (10^{-8}–10^{-6} M) in the reaction mixture caused a significant decrease in RNA synthesis activity in the nuclei isolated from normal rat

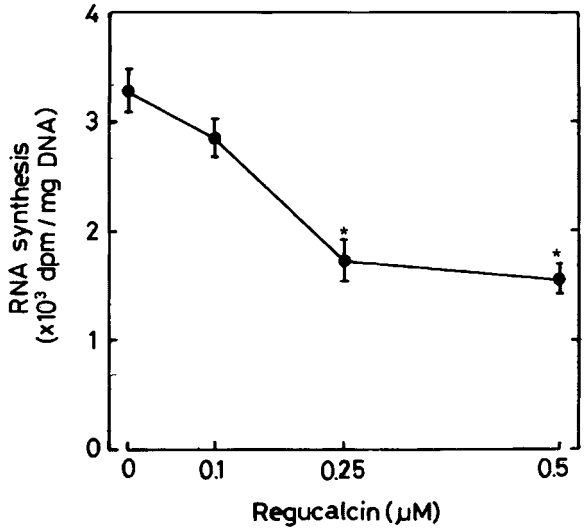

Fig. 1. Effect of regucalcin addition on RNA synthesis activity in the nucleus of normal rat liver. Nuclear RNA synthesis was measured by the incorporation of [^{3}H]-UTP into the nuclear RNA in the presence of either vehicle or exogenous regucalcin (0.1, 0.25, or 0.5 μM) in the reaction mixture. Each value is the mean ± SEM of six rats. *$P < 0.01$, compared with the control (none).

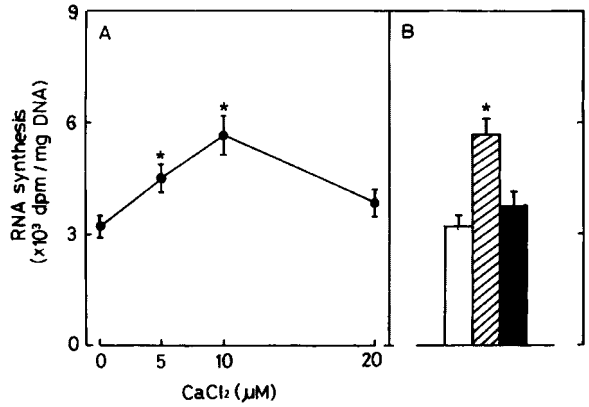

Fig. 3. Effect of calcium addition on RNA synthesis activity in the nucleus of normal rat liver. **A**: Nuclear RNA synthesis was measured by the incorporation of [^{3}H]-UTP into the nuclear RNA in the presence of either vehicle or $CaCl_2$ (5, 10, or 20 μM). **B**: The reaction mixture contained either vehicle, $CaCl_2$ (10 μM) or $CaCl_2$ (10 μM) plus regucalcin (0.25 μM). Each value is the mean ± SEM of six rats. *$P < 0.01$, compared with the control (none) value. White bars, control (none); hatched bar, $CaCl_2$ addition; black bar, $CaCl_2$ plus regucalcin addition.

liver (Fig. 2A). In the presence of α-amanitin (10^{-6} M), regucalcin (0.25 μM) did not have a significant effect on nuclear RNA synthesis activity (Fig. 2B).

The effect of calcium chloride addition on RNA synthesis activity in the nuclei isolated for normal rat liver is shown in Figure 3. The reaction mixture contained either vehicle or calcium chloride (5, 10, or 20 μM). Nuclear RNA synthesis activity is significantly increased by the addition of calcium chloride (5 or 10 μM) (Fig. 3A). The effect of calcium addition was weakened by increasing concentration of calcium (20 μM). The effect of calcium (10 μM) addition in increasing nuclear RNA synthesis activity was completely prevented in the presence of regucalcin (0.5 μM) (Fig. 3B).

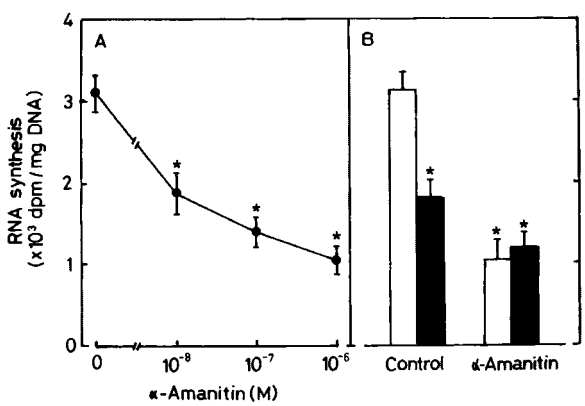

Fig. 2. Effect of α-amanitin on RNA synthesis activity in the nucleus of normal rat liver. **A**: Nuclear RNA synthesis was measured by the incorporation of [^{3}H]-UTP into the nuclear RNA in the presence of either vehicle or α-amanitin (10^{-8}–10^{-6} M) in the reaction mixture. **B**: The reaction mixture contained either vehicle or regucalcin (0.25 μM) in the absence or presence of α-amanitin (10^{-6} M). Each value is the mean ± SEM of six rats. *$P < 0.01$, compared with the control (none) value. White bars, control (none); black bars, regucalcin.

Enhancement of Nuclear RNA Synthesis Activity in Regenerating Rat Liver

Rats were partially hepatectomized, and 24, 48, and 72 h later the animals were killed by bleeding. Sham-operated rats were used as control at zero time after partial hepatectomy. The change in nuclear RNA synthesis activity after partial hepatectomy is shown in Figure 4. Nuclear RNA synthesis activity was significantly enhanced in regenerating rat liver after partial hepatectomy. This enhancement was significantly inhibited in the presence of either PD98059 (10^{-5} M), an inhibitor of MAPK kinase, staurosporine (10^{-6} M), an inhibitor of protein kinase C, or vanadate (10^{-3} M), an inhibitor of protein phosphatase in the reaction mixture.

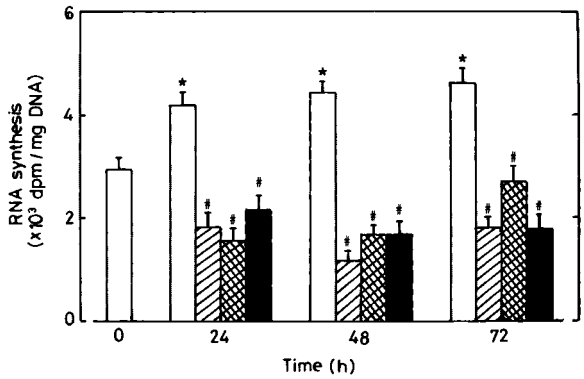

Fig. 4. Change in RNA synthesis activity in the nucleus of regenerating rat liver after partial hepatectomy. Rats were partially hepatectomized, and 24, 48, and 72 h later the animals were killed by bleeding. Nuclear RNA synthesis was measured by the incorporation of [^{3}H]-UTP into the nuclear RNA in the presence of either vehicle, PD98057 (10^{-5} M), staurosporine (10^{-6} M), or vanadate (10^{-3} M). Each value is the mean ± SEM of six rats. *$P < 0.01$, compared with the control value obtained at zero time after partial hepatectomy. #$P < 0.01$, compared with the control (none) none; hatched bars, PD98059; double hatched bars, staurosporine; black bar, vanadate.

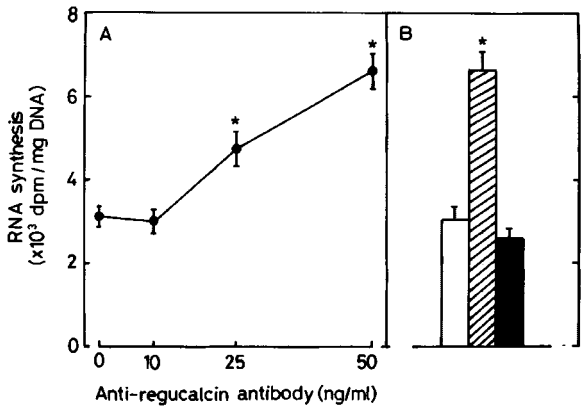

Fig. 6. Effect of anti-regucalcin monoclonal antibody on RNA synthesis activity in the nucleus of normal rat liver. **A**: Nuclear RNA synthesis was measured by the incorporation of [^{3}H]-UTP into the nuclear RNA in the presence of either vehicle or anti-regucalcin monoclonal antibody (10, 25, or 50 ng/ml). **B**: The reaction mixture contained either vehicle, anti-regucalcin monoclonal antibody (50 ng/ml), or the antibody (50 ng/ml) plus regucalcin (1.0 μM). Each value is the mean ± SEM of six rats. *$P < 0.01$, compared with the control (none) value. White bars, control (none); hatched bar, the antibody; black bar, the antibody plus regucalcin.

Increase in Nuclear Regucalcin Levels in Regenerating Rat Liver

The change in regucalcin levels in the nuclei of regenerating rat liver after partial hepatectomy is shown in Figure 5. Rats were killed at 24, 48, or 72 h after partial hepatectomy. Regucalcin levels were significantly enhanced in the nuclei of regenerating rat liver obtained at 24, 48, or 72 h after partial hepatectomy as compared with that of sham-operated rats.

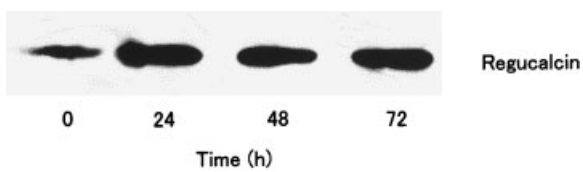

Fig. 5. Analysis of regucalcin protein in the nucleus of regenerating rat liver. Rats were partially hepatectomized, and 24, 48, and 72 h later the animals were killed by bleeding. Liver nuclei were isolated from the homogenate. Western blot analysis was carried out using the extracts (50 μg of nuclear proteins) obtained from the nucleus after partial hepatectomy. The figure shows one of five experiments with separate rats. The densitometric data showed 250 ± 11.5, 211 ± 15.6, and 240 ± 17.3 (percent of control; mean ± SEM of four rats) at 24, 48, or 72 h after partial hepatectomy. These data were significantly ($P < 0.01$) different as compared with the control (none) value.

Effect of Anti-Regucalcin Antibody on Nuclear RNA Synthesis Activity in Normal and Regenerating Rat Livers

The effect of anti-regucalcin monoclonal antibody on RNA synthesis activity in the nuclei of normal rat liver is shown in Figure 6. The presence of anti-regucalcin monoclonal antibody (25 or 50 ng/ml) in the reaction mixture caused a significant increase in RNA synthesis activity in the nuclei isolated from normal rat liver (Fig. 6A). The effect of anti-regucalcin monoclonal antibody (50 ng/ml) in increasing nuclear RNA synthesis was completely blocked by the addition of regucalcin (1 μM) (Fig. 6B).

The effect of anti-regucalcin monoclonal antibody on RNA synthesis activity in the nuclei of regenerating rat liver is shown in Figure 7. Anti-regucalcin monoclonal antibody (25 or 50 ng/ml) was added into the reaction mixture containing the nuclei of livers obtained at 24, 48, or 72 h after partial hepatectomy. The presence of anti-regucalcin monoclonal antibody (50 ng/ml) caused a significant increase in RNA synthesis activity in the nuclei of liver obtained at 24, 48, or 72 h after partial hepatectomy as compared with that of control without the antibody addition of each time point. The effect of anti-regucalcin monoclonal antibody (50 ng/ml) in increasing nuclear RNA synthesis activity was

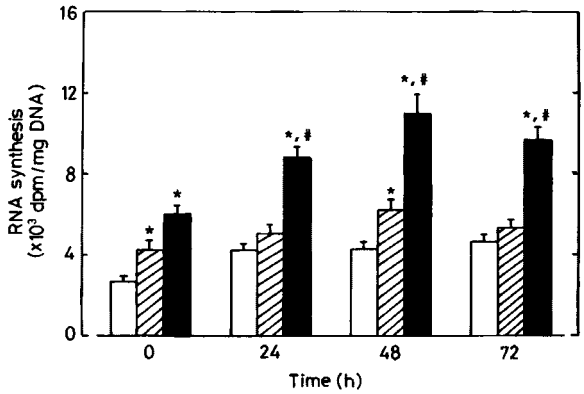

Fig. 7. Effect of anti-regucalcin monoclonal antibody on RNA synthesis activity in the nucleus of regenerating rat liver. Rats were partially hepatectomized, and 24, 48, and 72 h later the animals were killed by bleeding. Nuclear RNA synthesis was measured by the incorporation of [^{3}H]-UTP into the nuclear RNA in the presence of either vehicle or anti-regucalciun monoclonal antibody (25 or 50 ng/ml). Each value is the mean ± SEM of six rats. *$P < 0.01$, compared with the control (none) value. #$P < 0.01$, compared with the value with the antibody (50 ng/ml) addition obtained at zero time after partial hepatectomy. White bars, control (none); hatched bar, the antibody (25 ng/ml); black bar, the antibody (50 ng/ml).

significantly enhanced in the nuclei of regenerating liver at 24, 48, or 72 h after partial hepatectomy.

The effect of various inhibitors on the anti-regucalcin monoclonal antibody-increased nuclear RNA synthesis activity in regenerating rat liver is shown in Figure 8. Livers were obtained

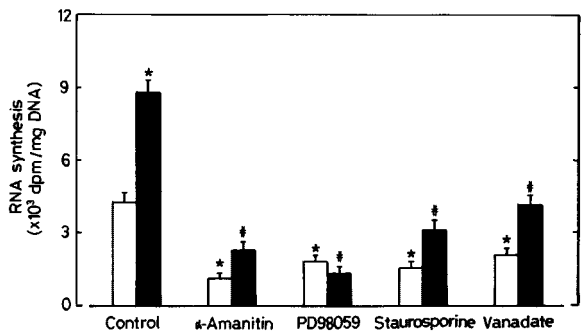

Fig. 8. Effect of various inhibitors on RNA synthesis activity in the nucleus of regenerating rat liver. Rats were partially hepatectomized, and 24 h later the animals were killed by bleeding. Nuclear RNA synthesis was measured by the incorporation of [^{3}H]-UTP into the nuclear RNA in the presence of either vehicle or anti-regucalciun monoclonal antibody (50 ng/ml) in the absence or presence of α-amanitin (10^{-6} M), PD98059 (10^{-5} M), staurosporin (10^{-6} M), or vanadate (10^{-3} M). Each value is the mean ± SEM of six rats. *$P < 0.01$, compared with the control (none) value. #$P < 0.01$, compared with the value obtained from anti-regucalcin monoclonal antibody addition. White bars, without the antibody; black bar, with the antibody.

at 24 h after partial hepatectomy. Liver nuclear RNA synthesis activity was measured in the reaction mixture containing either vehicle, α-amanitin (10^{-6} M), PD98059 (10^{-5} M), staurosporine (10^{-6} M), or vanadate (10^{-3} M) without or with anti-regucalcin monoclonal antibody (50 ng/ml). Nuclear RNA synthesis activity without or with the antibody addition was significantly decreased in the presence of α-amanitin, PD98059, staurosporine, or vanadate.

DISCUSSION

Regucalcin is greatly present in the liver of rats [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993] and the protein has been shown to translocate from cytoplasm to nucleus of liver cells [Omura and Yamaguchi, 1999a; Tsurusaki et al., 2000]. Regucalcin has been demonstrated to inhibit the activity of Ca^{2+}-dependent protein kinases [Katsumata and Yamaguchi, 1998], protein phosphatases [Omura and Yamaguchi, 1999b], and DNA synthesis [Tsurusaki and Yamaguchi, 2002] in the nuclei of normal and regenerating rat liver, suggesting that regucalcin plays an important role in the regulation of nuclear function in liver cells. Moreover, the present study clearly demonstrates that regucalcin has an inhibitory effect on RNA synthesis activity in the nuclei of normal rat liver, and that the endogenous protein plays a suppressive role in the enhancement of nuclear RNA synthesis activity in regenerating rat liver with proliferative cells after partial hepatectomy. The finding further supports the view that regucalcin plays a regulatory role in liver nuclear function.

The effect of regucalcin in decreasing nuclear RNA synthesis activity in normal rat liver was not seen in the presence of α-amanitin, an inhibitor of RNA polymerase II and III. This result suggests that the effect of regucalcin on nuclear RNA synthesis activity is partly resulted from its inhibitory action on RNA polymerase II and III. Meanwhile, it has been reported that Ca^{2+} has a stimulatory effect on RNA synthesis in liver nucleus [Pardo and Fernandez, 1982; Yamaguchi and Ueoka, 1997]. This effect may be partly mediated through Ca^{2+}-dependent protein kinase [Pardo and Fernandez, 1982]. The stimulatory effect of Ca^{2+} addition on nuclear RNA synthesis activity was completely blocked in the presence of regucalcin. It has been shown that regucalcin

can inhibit Ca^{2+}-dependent protein kinases in rat liver nucleus [Katsumata and Yamaguchi, 1998]. Presumably, the effect of regucalcin in decreasing RNA synthesis activity in liver nucleus is partly involved in its inhibitory action on the activities of both RNA polymerase II and III and Ca^{2+}-dependent protein kinases. Further mechanism remains to be elucidated.

Nuclear RNA synthesis activity was significantly enhanced in regenerating rat liver with proliferative cells after partial hepateactomy. This enhancement was significantly inhibited in the presence of PD98059, an inhibitor of MAPK kinase [Zhang et al., 1999], staurosporine, an inhibitor protein kinase C [Tamaoki et al., 1986], and vanadate, an inhibitor of protein phosphatase [Hunter, 1995]. This finding suggests that the enhancement of nuclear RNA synthesis activity in regenerating rat liver is partly dependent on the activation of protein kinases and protein phosphatases, which are involved intracellular signaling pathway of hormone and growth factors.

It has been reported that regucalcin mRNA expression is enhanced in regenerating rat liver obtained at 24, 48, and 72 h after partial hepatectomy [Yamaguchi and Kanayama, 1995]. Regucalcin protein was found to be increased in the nuclei of regenerating rat liver after partial hepatectomy, suggesting a role of endogenous regucalcin in the nucleus. The presence of anti-regucalcin monoclonal antibody in the reaction mixture caused a significant increase in RNA synthesis activity in the nuclei of normal rat liver. This increase was completely abolished by the addition of regucalcin. The effect of anti-regucalcin monoclonal antibody in increasing nuclear RNA synthesis activity in regenerating rat liver was significantly enhanced in the presence of the antibody in the reaction mixture. This finding suggests that endogenous regucaclin, which is increased in the nuclei of regenerating liver, plays a suppressive role in the enhancement of nuclear RNA synthesis activity.

The effect of anti-regucalcin monoclonal antibody in enhancing RNA synthesis activity in the nuclei of regenerating rat liver was markedly inhibited in the presence of α-amanitin, PD98059, staurosporine, or vanadate in the reaction mixture. From this result, it is speculated that the enhancement of nuclear RNA synthesis activity in regenerating liver is partly suppressed through signaling pathways that endogenous regucalcin inhibits RNA polymerase II and III, MAPK kinase, protein kinase C, and protein phosphatases in the liver nucleus. Thus the mechanism by which regucalcin inhibits nuclear RNA synthesis activity in proliferating liver cells, may be complex.

Regucalcin could inhibit RNA synthesis activity in the nuclei of normal and regenerating rat livers. Regucalcin may have a role as the transcription-related factor in liver nucleus. Whether regucalcin can directly bind to the promotor region in the gene is unknown, however.

In conclusion, it has been demonstrated that endogenous regucalcin is increased in the nuclei of regenerating rat liver with proliferative cells after partial hepatectomy, and that the protein has a suppressive effect on the enhancement of nuclear RNA synthesis activity in regenerating liver cells. This finding further supports the view that regucalcin may play a role in the regucalcin of nuclear function in liver cells.

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