Journal of Cellular Biochemistry 74:81–89 (1999)

Involvement of Intracellular Signaling Factors in the Serum-Enhanced Ca^{2+}-Binding Protein Regucalcin mRNA Expression in the Cloned Rat Hepatoma Cells (H4-II-E)

Masayoshi Yamaguchi* and Michiko Nakajima

Laboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka, Shizuoka City 422-8526, Japan

Abstract The involvement of signaling factors, which are related to serum component, on the regucalcin mRNA expression in the cloned rat hepatoma cells (H4-II-E) was investigated. The change in regucalcin mRNA levels was analyzed by Northern blotting using rat liver regucalcin complementary DNA (0.9 kb of open reading frame). H4-II-E cells were cultured for 2 or 6 h in a medium containing various reagents in the presence of serum (10% fetal bovine serum) after the subconfluent with 3-day-culture. The regucalcin mRNA expression was significantly increased by serum addition. This increase was clearly inhibited by the presence of EGTA (10^{-3} M), A23187 (10^{-6} M), trifluoperazine (10^{-5} M), staurosporine (10^{-7} M), or genistein (10^{-5} M) with 6-h-culture, although the β-actin mRNA expression was not altered by the reagents. Meanwhile, the regucalcin mRNA expression was significantly stimulated by the addition of Bay K 8644 (2.5×10^{-6} M) in the presence of serum. This effect was also seen in the presence of genistein (10^{-5} M). The present study suggests that the regucalcin mRNA expression is mediated through signaling pathways which are partly involved in Ca^{2+}-dependent protein kinases and tyrosine kinase in H4-II-E hepatoma cells. J. Cell. Biochem. 74:81–89, 1999. © 1999 Wiley-Liss, Inc.

Key words: regucalcin; calcium signaling; protein kinase C; tyrosine kinase; gene expression; hepatoma cells

Calcium ion (Ca^{2+}) plays a role as an important second messenger signal in a variety of pathways to produce a Ca^{2+}-mediated physiological responses in many cells [Cheung, 1980; Williamson et al., 1981; Bygrave and Benedetti, 1993; Kraus-Friedman and Feng, 1996]. The Ca^{2+} signal is transmitted to an intracellular response partly via a family of Ca^{2+}-binding protein [Heizman, 1991]. A novel Ca^{2+}-binding protein regucalcin has been proposed to play a role as a regulatory protein of Ca^{2+} signaling; the protein has an inhibitory effect on Ca^{2+}/calmodulin-dependent enzyme activation and protein kinase C activation [Yamaguchi and Tai, 1991; Yamaguchi and Mori, 1990; Katsumata and Yamaguchi, 1998].

The regucalcin gene is localized on rat chromosome Xq11.1-12 proximal end [Shimokawa et al., 1995]. Regucalcin mRNA and its protein are expressed in the liver and kidney cortex of rats [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993], and the expressions are stimulated by the administration of calcium chloride to rats [Shimokawa and Yamaguchi, 1992; Isogai and Yamaguchi, 1995]. Recently, it has been shown that calcium administration stimulates the binding of AP-1 factor to the 5′-flanking region of the rat gene for regucalcin [Murata and Yamaguchi, 1998a,b]. Moreover, it has been demonstrated that the 5′-flanking region of the rat regucalcin gene ligated to the luciferase reporter gene possesses promotor activity in H4-II-E hepatoma cells, and that the existence of *trans*-acting factors responsible for Ca^{2+}, cyclic AMP, phorbol ester, and insulin responses of the rat regucalcin gene [Murata and Yamaguchi, 1999]. Thus, the

Grant sponsor: Scientific Research from the Ministry of Education, Science, and Culture, Japan; Grant number: 10672048.

*Correspondence to: Masayoshi Yamaguchi, Ph.D., Laboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka City 422-8526, Japan.

Received 24 November 1998; Accepted 21 January 1999

© 1999 Wiley-Liss, Inc.

promoter characterization of the rat gene for regucalcin which is related to transcriptional regulation by various signaling factors is clarified.

The present study was undertaken to determine the effect of serum components on the regucalcin mRNA expression in the cloned H4-II-E hepatoma cells. Serum contains various hormones, growth factors, and cytokines which may stimulate transcriptional activity in H4-II-E cells. It is unknown, however, whether the regucalcin mRNA expression is mediated through the serum responsible intracellular factors related to signal transduction in H4-II-E cells. We used various inhibitors of protein kinases in this study. It was found that the serum-stimulated regucalcin mRNA expression might be partly involved in Ca^{2+}/calmodulin-dependent protein kinase, protein kinase C, and tyrosine kinase.

MATERIALS AND METHODS

Chemicals

Deoxycytidine 5′-[α-^{32}P] triphoshate ([^{32}P] dCTP); 110 Tbq/mmol and nylon membranes (Hybond N$^+$) for Northern hybridization were obtained from Amersham (Buckinghamshire, England). A human β-actin gene fragment (0.43 kb) as an internal standard was obtained from Wako Pure Chemical Co. (Osaka, Japan). Molecular-sized standard (0.24–9.5 kb RNA ladder) for electrophoresis of RNA was purchased from Bethesda Research Laboratories (Gaithersburg, MD). Trifluoperazine dimalate (TFP), staurosporine (SP), dibucaine, genistein, cycloheximide, actinomycin D, ethyleneglycol-bis-(aminoethylether) N, N, N′, N′-tetraacetic acid (EGTA), and A23187 were obtained from Sigma Chemical Co. (St. Louis, MO). S (−)-Bay K 8644 from Research Biochemicals International (Natick, MA) was dissolved in dimethyl sulfoxide (DMSO) to a stock solution of 5 mM and then diluted in medium to give the desired drug concentration. Fetal bovine serum, α-minimum essential medium (α-MEM), penicillin, and streptomycine were obtained from Gibco-BRL (Grand Island, NY). Other reagents were purchased from Wako Pure Chemical Co. Any water and solutions used for RNA preparation were treated with chemical diethylpyrocarbonate (DEPC, Sigma) to inhibit RNase activity.

Cell Culture

H4-II-E hepatoma cells (1×10^6) were maintained for 3 days in α-MEM supplemented with 5 mM glucose, 10% heat-inactivated fetal bovine serum (FBS), 50 units/ml penicillin, and 50 μg/ml streptomycin in humidified 5% CO_2/95% air at 37°C to obtain confluent monolayers. For experiments, after medium change the cells were cultured for 2 or 6 h in a medium containing either vehicle (0.2% bovine serum albumin) or 10% FBS in the absence or presence of various drugs.

Isolation of RNA

Total RNAs from H4-II-E hepatoma cells were prepared as described [Chomozynshi and Sacchi, 1987]. Hepatoma cells were homogenized in buffer solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarcosyl, 0.1 M 2-mercaptoethanol, and 2 M

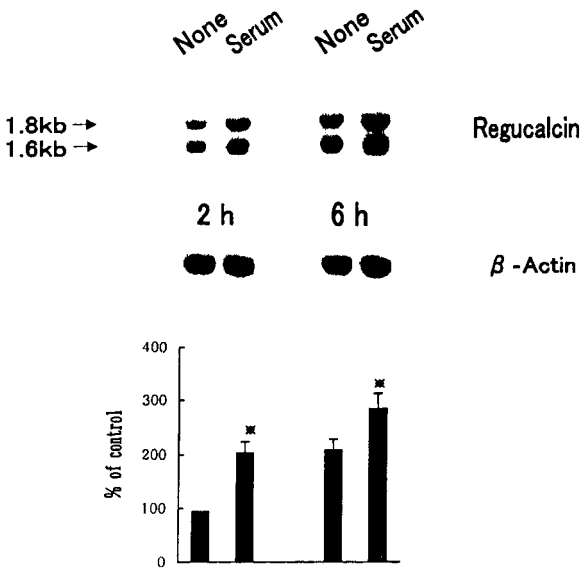

Fig. 1. Effect of serum addition on the regucalcin mRNA expression in H4-II-E hepatoma cells. The cells were cultured for 2 or 6 h in a medium containing either vehicle (0.2% bovine serum albumin) or serum (10% FBS) after the subconfluents with 3-day-culture. Total RNAs (20 μg) extracted from the cells were subjected to the hybridizing analysis. β-Actin mRNA levels were not appreciably altered by serum addition. The arrowhead indicates hybridizing bands corresponding the mRNA encoding regucalcin. Each bar represents densitometric data (% of control obtained from 2-h-culture without serum; mean ± S.E.M.) of regucalcin mRNA levels for each of the time points with culture. The figure shows one of four experiments with separate cell culture. *$P < 0.01$ as compared with the value without serum addition.

sodium acetate. Total RNAs were extracted by vigorous shaking in a mixture of phenol, chloroform, and isoamylalcohol, and phases were separated by centrifugation at 10,000*g* for 20 min at 4°C. RNA located in the aqueous phase was precipitated with isopropanol at −20°C. RNA precipitates were pelleted by centrifugation, and the pellets were redissolved in 50 μl of DEPC-treated 0.5% sodiumdodecyl sulfate (SDS).

Northern Blotting

Twenty micrograms of total RNAs extracted from hepatoma cells was electrophoresed in 1.2% agarose denaturing gels containing 2.2 M formaldehyde in MOPS buffer (pH 7, containing 20 mM 3-N-morpholinopropanesulfonic acid, 5 mM sodium acetate, and 1 mM EDTA) with 3 V/cm^3 for 3 h [Sambrook et al., 1989]. The electrophoresed gels were transferred to nylon membranes by blotting [Shimokawa and Yamaguchi, 1992]. A part of regucalcin cDNA (the 0.6-kb, KpnI-PstI insert) was labeled with [^{32}P]dCTP by random primers with the DNA polymerase Klenow fragment. This radioactive probe was used for hybridization detection of RNAs on blots. The membranes were prehybridized and hybridized in buffer solution containing 50% formamide, 5 × SSPE (1 × SSPE; 1.15 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA), 5 × Denhardt's reagent; 0.02% w/v each of bovine serum albumin [BSA], Ficoll and polyvinylpyrrolidine) and 0.5% SDS with ^{32}P-labeled regucalcin cDNA in a sealed plastic bag at 42°C for 16 h. After hybridization, the membranes were washed as follows: 2 × SSPE and 0.1% SDS at

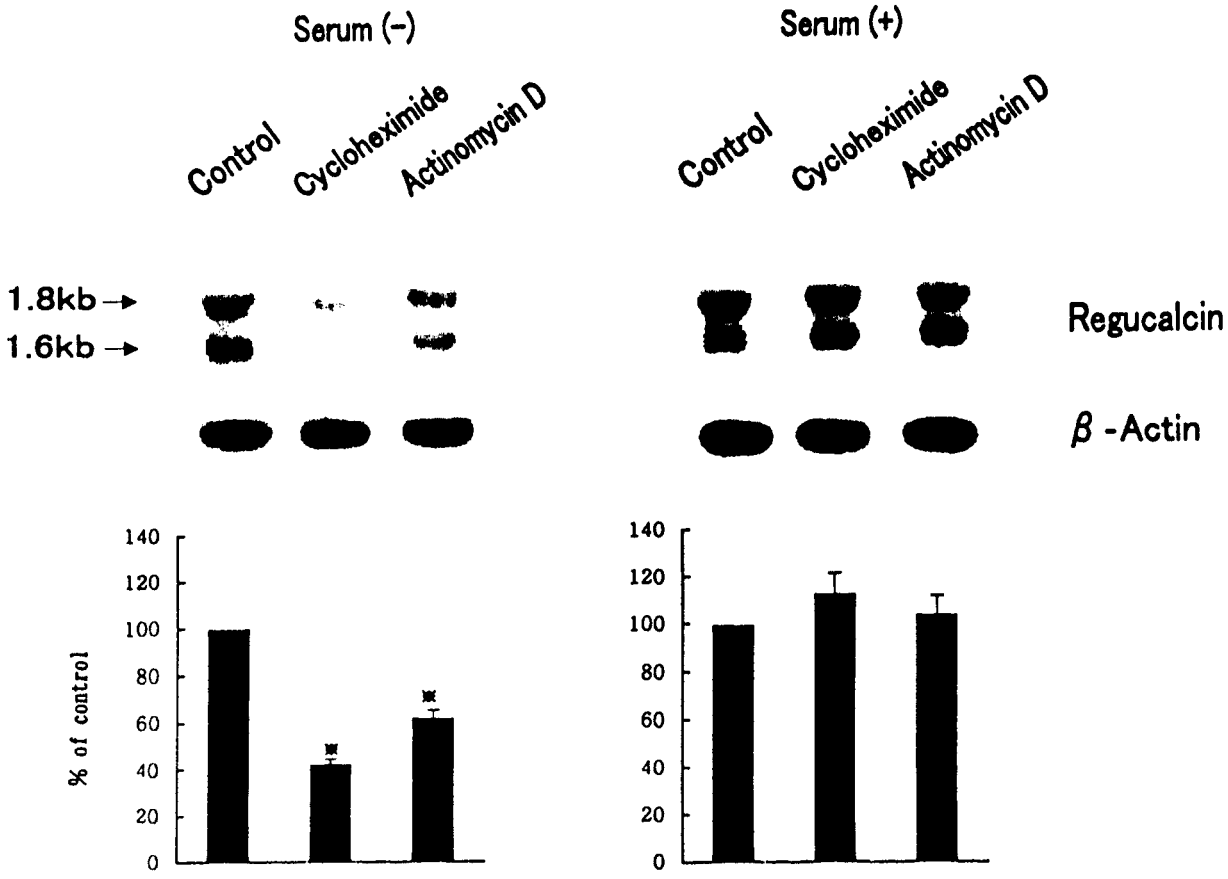

Fig. 2. Effect of cycloheximide or actinomycin D on the regucalcin mRNA expression in H4-II-E hepatoma cells. The cells were cultured for 6 h in a medium containing either vehicle (0.2% bovine serum albumin) or serum (10% FBS) in the absence or presence of cycloheximide (10^{-6} M) or actinomycin D (10^{-7} M) after the subconfluents with 3-day-culture. Total RNAs (20 μg) extracted from the cells were subjected to the hybridizing analysis. β-Actin mRNA levels were not appreciably altered by the addition of cycloheximide or actinomycin D. The arrowhead indicates hybrizing bands corresponding to mRNA encoding regucalcin. Each bar represents densitometric data (% of control; mean ± S.E.M.) for regucalcin mRNA levels. The figure shows one of four experiments with separate cell culture. *$P < 0.01$ as compared with the control value.

42°C (twice, each for 15 min), followed by 0.1 × SSPE and 0.1% SDS at room temperature (twice, each for 15 min), and then the membranes were usually exposed to X-ray film for 48 h.

The quantity and integrity of mRNA were monitored by rehybridizing with a radioactive cDNA probe from human β-actin gene fragment under identical conditions. No noticeable change in the level of RNA hybridized with the β-actin probe was observed throughout the present experiments. The size of the hybridizing RNA was determined by running the standard RNA molecules of known sizes in parallel. The density of the autoradiographic data was quantified by densitometer scanning (Dual-wavelength Flying-spot Scanner, CS-9000, Shimadzu Co., Japan).

Statistical Methods

The significance of the difference between the values was estimated by Student's *t*-test or by analysis of variance (ANOVA) for comparing multiple groups. *P*-values of <0.05 were considered to indicate a statistically significant difference.

RESULTS

The involvement of signaling factors which are related to serum component in the expression of the regucalcin mRNA in H4-II-E hepatoma cells was examined. When the cells were cultured for 2 or 6 h in a medium containing either vehicle or serum (10% FBS) after the subconfluents with 3-day-culture, the regucalcin mRNA levels were significantly increased by the addition of serum (Fig. 1). In the cell extracts, two distinct mRNA isoforms of 1.8 and 1.6 kb, which are though to be generated by alternative splicing of common RNA precursor molecules [Shimokawa and Yamaguchi, 1992], were seen. Meanwhile, the β-actin mRNA levels were markedly expressed in H4-II-E cells,

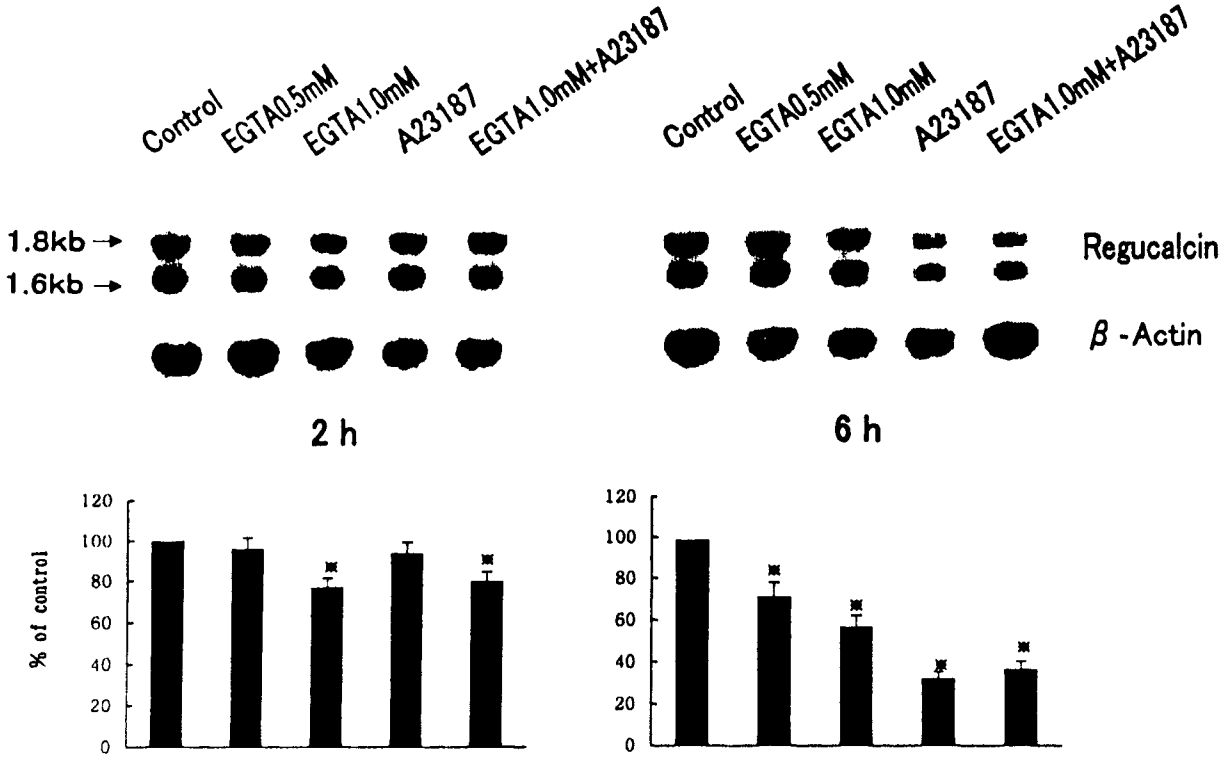

Fig. 3. Effect of EGTA or A23187 on the regucalcin mRNA expression in H4-II-E hepatoma cells. The cells were cultured for 2 or 6 h in a medium containing either vehicle, EGTA (0.5 or 1.0 mM), A23187 (10^{-6} M) or EGTA (1.0 mM) plus A23187 (10^{-6} M) in the presence of serum (10% FBS) after the subconfluents with 3-day-culture. Total RNAs (20 μg) extracted from the cells were subjected to the hybridizing analysis. β-Actin mRNA levels were not appreciably altered by EGTA or A23187 addition. The arrowhead indicates hybridizing bands corresponding to mRNA encoding regucalcin. Each bar represents densitometric data (% of control; mean ± S.E.M.) for regucalcin mRNA levels. The figure shows one of four experiments with separate cell culture. $^*P < 0.01$ as compared with the control value.

and the levels were not appreciably altered by serum addition.

The effect of cycloheximide or actinomycin D on the regucalcin mRNA expression in H4-II-E hepatoma cells is shown in Figure 2. The cells were cultured for 6 h in a medium containing either vehicle or serum (10% FBS) in the absence or presence of cycloheximide (10^{-6} M) or actinomycin D (10^{-7} M) after the subconfluents with 3-day-culture. In the absence of serum, the regucalcin mRNA levels were significantly decreased by the addition of cycloheximide or actinomycin D. Such an effect, however, was not seen in the presence of serum. Meanwhile, the β-actin mRNA levels were not appreciably altered by the addition of cycloheximide or actinomycin D. Thus, serum addition had a potent effect.

The effect of EGTA or A23187 on the regucalcin mRNA expression in H4-II-E hepatoma cells is shown in Figure 3. The cells were cultured for 2 or 6 h in a medium containing either vehicle, EGTA (0.5 or 1.0 mM), A23817 (10^{-6} M), or EGTA (1.0 mM) plus A23187 (10^{-6} M) in the presence of serum (10% FBS) after the subconfluents with 3-day-culture. In the culture of 2 h, the regucalcin mRNA levels were significantly decreased by the addition of EGTA (1.0 mM) or EGTA (1.0 mM) plus A23187 (10^{-6} M), while EGTA (0.5 mM) or A23187 (10^{-6} M) alone had no effect. However, the culture of 6 h caused a significant decrease in the regucalcin mRNA levels in the presence of EGTA (0.5 and 1.0 mM), A23187 (10^{-6} M) or EGTA (1.0 mM) plus A23187 (10^{-6} M). This significant decrease was also recognized when the data was normalized against β-actin mRNA values (data not shown).

The effect of various protein kinase inhibitors on the regucalcin mRNA expression in H4-II-E hepatoma cells is shown in Figure 4. The cells were cultured for 2 and 6 h in a medium containing either vehicle, trifluoperazine (TFP; 10^{-5} M), staurosporin (SP; 10^{-7} M), dibucaine (10^{-4} M), or genistein (10^{-5} M) after the subconfluent with 3-day-culture. In the culture of 2 h, the regucalcin mRNA levels were significantly decreased by the addition of TFP, SP, or dibucaine, while genistein had no effect. The 6-h-

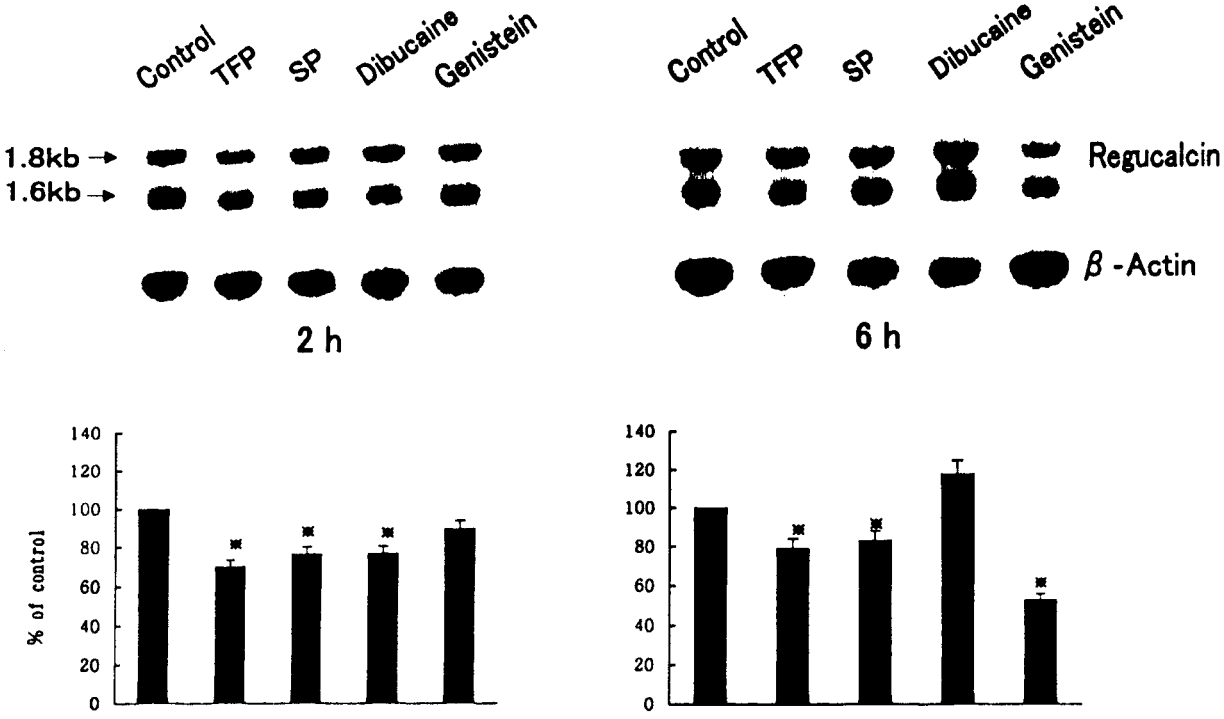

Fig. 4. Effect of various inhibitors for signal transduction on the regucalcin mRNA expression in H4-II-E hepatoma cells. The cells were cultured for 2 or 6 h in a medium containing either vehicle, trifluoperazine (TFP; 10^{-5} M), staurosporine (10^{-7} M), dibucaine (10^{-4} M), or genistein (10^{-5} M) in the presence of serum (10% FBS) after the subconfluents with 3-day-culture. Total RNAs (20 μg) extracted from the cells were subjected to the hybridizing analysis. β-Actin mRNA levels were not appreciably altered by inhibitor addition. The arrowhead indicates hybridizing bands corresponding to mRNA encoding regucalcin. Each bar represents densitometric data (% of control; mean ± S.E.M.) for regucalcin mRNA levels. The figure shows one of four experiments with separate cell culture. *$P < 0.01$ as compared with the control value.

culture with genistein, however, caused a significant decrease in the regucalcin mRNA levels. Also, TFP and SP had a significant effect on the mRNA levels in the culture of 6 h. Meanwhile, the β-actin mRNA expression was not appreciably altered by the addition of various protein kinase inhibitors.

The effect of increasing concentrations of genistein on the regucalcin mRNA expression in H4-II-E hepatoma cells is shown in Figure 5. The cells were cultured for 6 h in a medium containing either vehicle or genistein (10^{-7} to 10^{-5} M) after the subconfluent of 3-day-culture. The regucalcin mRNA levels were significantly decreased by the presence of genistein (10^{-6} and 10^{-5} M). The concentration of 10^{-7} M had no effect. The β-actin mRNA levels were not appreciably altered by genistein addition.

The effect of Bay K 8644, a Ca^{2+}-channel agonist, on the regucalcin mRNA expression in H4-II-E hepatoma cells is shown in Figure 6. The cells were cultured for 2 or 6 h in a medium containing either vehicle, genistein (10^{-5} M), Bay K 8644 (2.5×10^{-6} M) or genistein (10^{-5} M) plus Bay K 8644 (2.5×10^{-6} M) after the subconfluent with 3-day-culture. The culture with Bay K 8644 for 2 and 6 h caused a significant increase in the regucalcin mRNA levels. The effect of Bay K 8644 in increasing the regucalcin mRNA levels was also seen in the presence of genistein, although genistein had a suppressive effect on the mRNA levels. Meanwhile, the β-actin mRNA expression was not appreciably altered by the addition of Bay K 8644 plus genistein.

DISCUSSION

A Ca^{2+}-binding protein regucalcin is mainly present in the cytoplasm of liver cells [Yamaguchi and Isogai, 1993]. The promoter character-

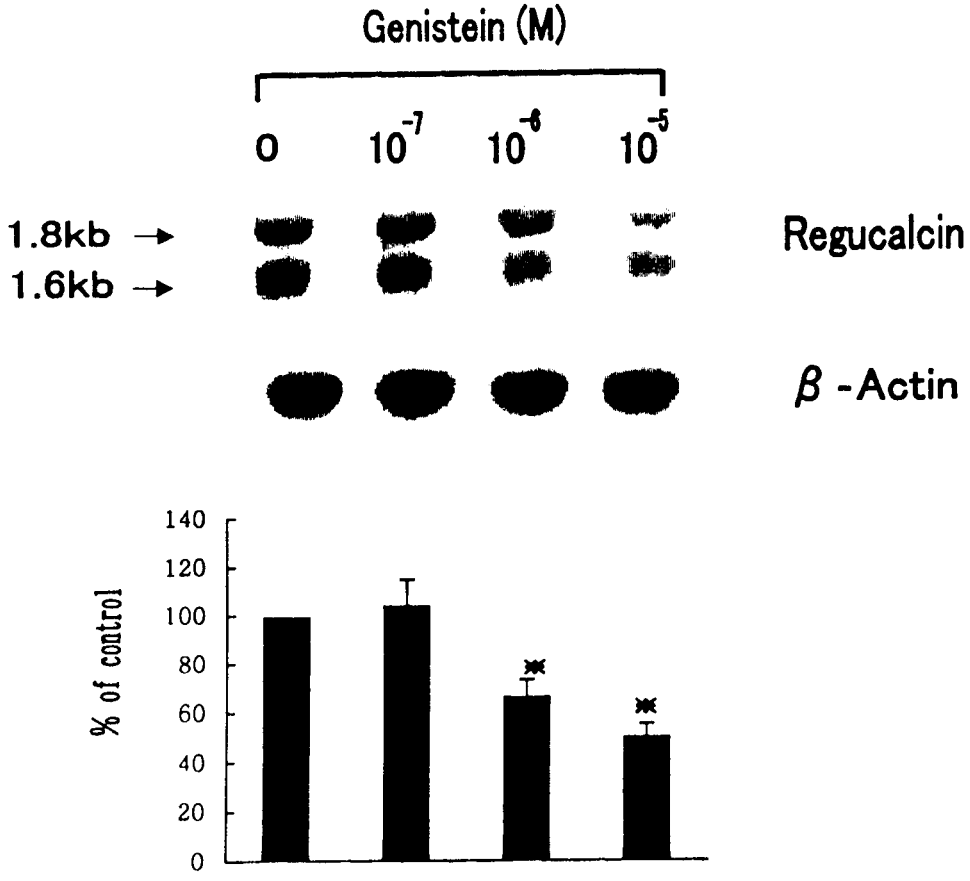

Fig. 5. Effect of genistein on the regucalcin mRNA expression in H4-II-E hepatoma cells. The cells were cultured for 6 h in a medium containing either vehicle or genistein (10^{-7} to 10^{-5} M) in the presence of serum (10% FBS) after the subconfluents with 3-day-culture. Total RNAs (20 μg) extracted from the cells were subjected to the hybridizing analysis. β-Actin mRNA levels were not appreciably hybridizing bands corresponding to mRNA encoding regucalcin. Each bar represents densitometric data (% of control; mean ± S.E.M.) for regucalcin mRNA levels. The figure shows one of four experiments with separate cell culture. *$P < 0.01$ as conpared with the control value.

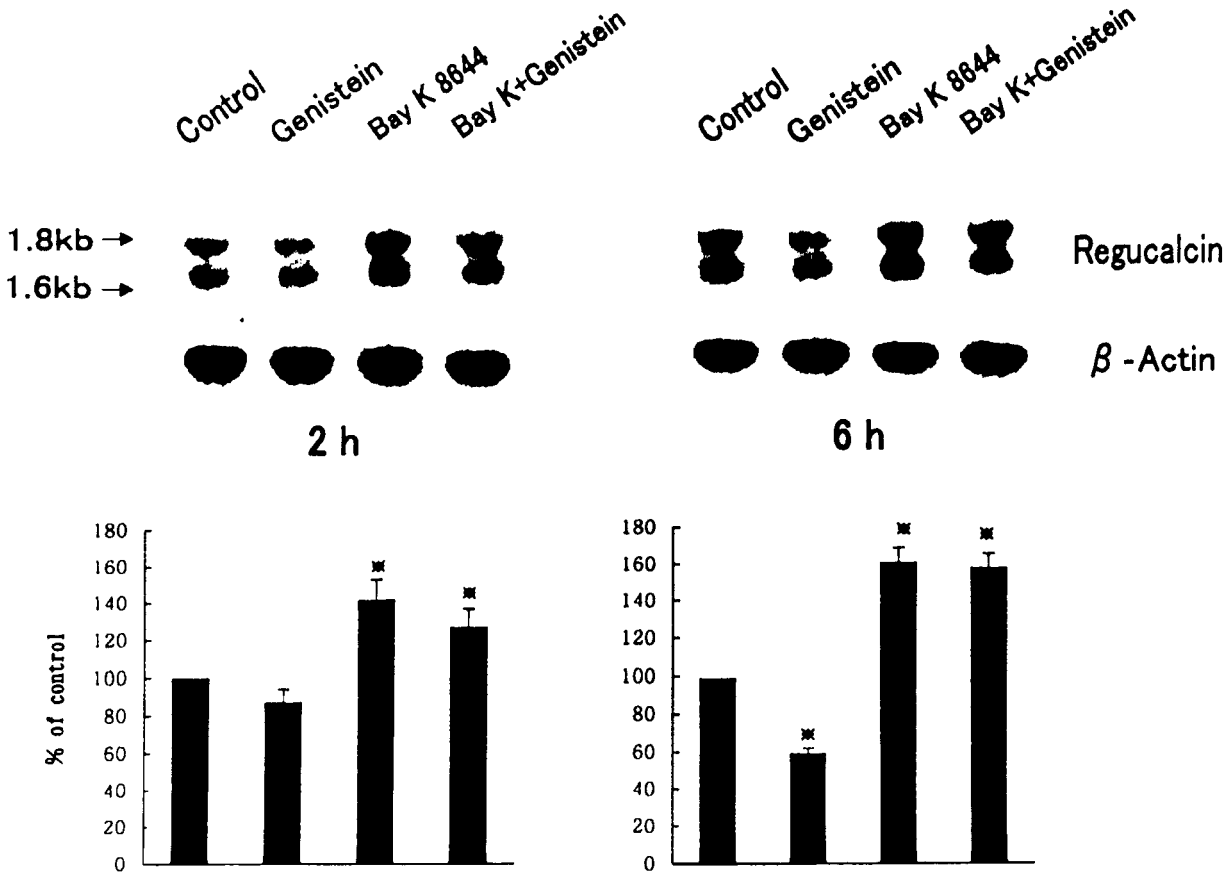

Fig. 6. Effect of Bay K 8644, a Ca^{2+}-channel agonist, on the regucalcin mRNA expression in the presence of genistein in H4-II-E hepatoma cells. The cells were cultured for 2 or 6 h in a medium containing either vehicle, genistein (10^{-5} M), Bay K 8644 (2.5×10^{-6} M), or Bay K 8644 (2.5×10^{-6} M) plus genistein (10^{-5} M) in the presence of serum (10% FBS) after the subconfluents with 3-day-culture. Total RNAs (20 μg) extracted from the cell were subjected to the hybridizing analysis. β-Actin mRNA levels were not appreciably altered by Bay K 8644 and genistein. The arrowhead indicates hybridizing bands corresponding to mRNA encoding regucalcin. Each bar represents densitometric data (% of control; mean ± S.E.M.) for regucalcin mRNA levels. The figure shows one of four experiments with separate cell culture. $*P < 0.01$ as compared with the control value.

ization of the rat gene for regucalcin which is related to transcriptional regulation by signaling factors has been clarified [Murata and Yamaguchi, 1999]. The intracellular signaling factors in stimulating the regucalcin mRNA expression in liver cells, however, remains to be elucidated. The present study was undertaken to clarify an involvement of the intracellular signaling factors which induces the stimulatory effect with serum components on regucalcin mRNA expression in the cloned H4-II-E hepatoma cells.

The regucalcin mRNA expression in H4-II-E hepatoma cells was significantly stimulated by culture with serum addition. This stimulation was also seen in the presence of cycloheximide, an inhibitor of protein synthesis, or actinomycin D, an inhibitor of transcriptional activity, although these inhibitors with the same concentrations caused a remarkable decrease in regucalcin mRNA expression in H4-II-E hepatoma cells without serum addition. This result may indicate that higher concentration is required to reveal inhibitor's effect in decreasing transcriptional activity in the presence of serum. Serum stimulation affecting the regucalcin mRNA expression may be potent. Presumably, the regucalcin mRNA expression may be partly mediated through a newly synthesized protein component in the cells.

The persence of EGTA, a chelator of Ca^{2+}, or A23187, an ionophore of Ca^{2+}, in the culture medium containing serum, caused a significant decrease in the regucalcin mRNA expression in H4-II-E hepatoma cells. In the presence of EGTA, the regucalcin mRNA expression was

not further suppressed by the addition of A23187. The effect of EGTA on the regucalcin mRNA expression may be based on the chelating of Ca^{2+}. A23187 may stimulate the release of Ca^{2+} from the cells. Presumably, the serum-stimulated regucalcin mRNA expression is partly mediated through Ca^{2+} signaling. Meanwhile, Bay K 8644, a Ca^{2+}-channel agonist, stimulated the regucalcin mRNA expression in the hepatoma cells, supporting the view that the expression is partly related to Ca^{2+} signaling.

Trifluoperazine and dibucaine are antagonists of Ca^{2+}/calmodulin-dependent protein kinase [Vincenzi, 1982]. Staurosporin is an inhibitor of protein kinase C [Tamaoki et al., 1986]. Genistein is an inhibitor of thyrosine kinase [Lin et al., 1994]. These inhibitors had a suppressive effect on the serum-stimulated regucalcin mRNA expression in H4-II-E hepatoma cells. The serum effect may be mediated through a signaling process which is related to Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C in H4-II-E hepatoma cells. Moreover, the regucalcin mRNA expression may be stimulated by signaling mechanism which is mediated through tyrosine kinases. Insulin has been shown to stimulate the regucalcin mRNA expression in the cloned hepatoma cells [Murata et al., 1997].

The effect of genistein in decreasing the regucalcin mRNA expression in H4-II-E hepatoma cells disappeared in the presence of Bay K 8644, a Ca^{2+}-channel agonist, in a medium containing serum. This finding suggests that the regucalcin mRNA expression is stimulated through the mechanism of Ca^{2+} action independent on the signaling process of tyrosine kinase. Presumably, the regucalcin mRNA expression is mediated through the signaling mechanism of the process of both Ca^{2+}-dependent protein kinase and tyrosine kinase in H4-II-E hepatoma cells. More recently, it has been shown that Ca^{2+} treatment stimulates the rat regucalcin gene expression through *cis*-acting elements in the 5′-flanking sequence of the gene, and that the existence of liver nuclear protein components which bind to the regucalcin gene is identified [Murata and Yamaguchi, 1998a,b]. It may be possible that this nuclear protein components which can bind to the 5′-flanking region of the regucalcin gene are phosphorylated by Ca^{2+} signaling-dependent protein kinases and/or tyrosine kinase.

In conclusion, it has been demonstrated that the regucalcin mRNA expression is mediated through a signaling pathway which is related to Ca^{2+}-dependent protein kinases and/or tyrosine kinase in H4-II-E hepatoma cells.

REFERENCES

Bygrave FL, Benedetti A. 1993. Calcium: its modulation in liver by crosstalk between the actions of glucagon and calcium-mobilizing agents. Biochem J 296:1–14.

Cheung WY. 1980. Calmodulin plays a pivotal role in cellular regulation. Science 202:19–27.

Chomozynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Heizmann CW, Hunziker W. 1991. Intracellular calcium-binding proteins: More sites than in sights. Trends Biochem Sci 16:98–103.

Isogai M, Yamaguchi M. 1995. Calcium administration increase calcium-binding protein regucalcin concentration in the liver of rats. Mol Cell Biochem 143:53–58.

Katsumata T, Yamaguchi M. 1998. Inhibitory effect of calcium-binding protein regucalcin on protein kinase activity in the nuclei of regenerating rat liver. J Cell Biochem 71:569–576.

Kraus-Friedman N, Feng L. 1996. The role of intracellular Ca^{2+} in the regulation of gluconeogenesis. Metabolism 45:389–403.

Liu Y, Bhalla K, Hill C, Priest DG. 1994. Evidence for involvement of tyrosine phosphorylation in taxol-induced apoptosis in a human ovarian tumor cell line. Biochem Pharmac 48:1265–1272.

Murata T, Shinya N, Yamaguchi M. 1997. Expression of calcium-binding protein regucalcin mRNA in the cloned human hepatoma cells (HepG2): stimulation by insulin. Mol Cell Biochem 175:163–168.

Murata T, Yamaguchi M. 1998a. Ca^{2+} administration stimulates the binding of AP-1 factor to the 5′-flanking region of the rat gene for the Ca^{2+}-binding protein regucalcin. Biochem J 329:157–163.

Murata T, Yamaguchi M. 1998b. Tissue-specific binding of nuclear factors to the 5′-flanking region of the rat gene for calcium-binding protein regucalcin. Mol Cell Biochem 178:305–310.

Murata T, Yamaguchi M. 1999. Promoter characterization of the rat gene for Ca^{2+}-binding protein regucalcin: transcriptional regulation by signaling factors. J Biol Chem 274:1277–1285.

Sambook J, Fritsch EF, Maniatis T. 1989. Northern hybridization. In: Molecular cloning, 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. p 7.39–7.52.

Shimokawa N, Matsuda Y, Yamaguchi M. 1995. Genomic cloning and chromosomal assignment of rat regucalcin gene. Mol Cell Biochem 151:157–163.

Shimokawa N, Yamaguchi M. 1992. Calcium administration stimulates the expression of calcium-binding protein regucalcin mRNA in rat liver. FEBS Lett 305:151–154.

Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M, Tomita E. 1986. Staurosporin, a potent inhibitor of phospholipid/Ca^{2+}-dependent protein kinase. Biochem Biophys Res Commum 135:397–402.

Vincenzi FF. 1982. Pharmacology of calmodulin antagonism. In: Gogfraind T, Albertini A, Paoletti R, editors. Calcium modulators. Amsterdam: Elsevier Biomedical Press. p 67–80.

Williamson JR, Cooper RH, Hoek JB. 1981. Role of calcium in the hormonal regulation of liver metabolism. Biochem Biophys Acta 639:243–295.

Yamaguchi M, Isogai M. 1993. Tissue concentration of calcium binding protein regucalcin in rats by enzyme-linked immunoadsorbent assay. Mol Cell Biochem 122: 68–68.

Yamaguchi M, Mori S. 1990. Inhibitiry effect of calcium-binding protein regucalcin on protein kinase C activity in rat liver cytosol. Biochem Med Metab Biol 43:140–146.

Yamaguchi M, Tai H. 1991. Inhibitory effect of calcium-binding protein regucalcin on Ca^{2+}/calmodulin-dependent cyclic nucleotide phosphodiesterase activity in rat liver cytosol. Mol Cell Biochem 106:25–30.