2036–2041 Nucleic Acids Research, 1994, Vol. 22, No. 11

A novel growth-inducible gene that encodes a protein with a conserved cold-shock domain

Kikukatsu Ito, Ken-ichi Tsutsumi[1], Takejiro Kuzumaki, Paul F.Gomez, Kaoru Otsu and Kiichi Ishikawa*

Department of Biochemistry, Yamagata University School of Medicine, Iida-nishi, Yamagata 990-23 and [1]Institute for Cell Biology and Genetics, Faculty of Agriculture, Iwate University, Ueda, Morioka, Iwate 020, Japan

Received March 7, 1994; Revised and Accepted April 22, 1994 DDBJ accession no. D28557

ABSTRACT

We have isolated a cDNA that encodes a novel member of the Y-box binding protein family, termed as RYB-a (Rat Y-box Binding protein-a). RYB-a is a 31 kDa protein that contains a conserved cold-shock domain and an amino acid alignment similar to those of charge zipper proteins. Expression of RYB-a mRNA was highly abundant in the skeletal muscle, spleen, and fetal liver. The expression is very low in new-born and adult livers, suggesting its expression is under developmental regulation. In addition, the expression of RYB-a mRNA was induced in the liver during regeneration and by stimulation of quiescent fibroblast cells with serum. Induction in the fibroblasts was inhibited by treating the cell with a specific tyrosine kinase inhibitor, genistein or by detachment of cell-adhesion. Since both treatments are known to inhibit G1 cells to enter S phase, RYB-a gene is thought to be a member of growth-inducible genes.

INTRODUCTION

DNA-binding proteins play central roles in cell growth. They are responsible for replicating the genome, for transcribing active genes, and for repairing damaged DNA. From the structural similarities, DNA-binding proteins can be divided into several classes, such as the helix–turn–helix proteins (1), the homeodomains (2,3), and the zinc finger proteins (4). Recently, Y-box binding factors have been shown to be a class of proteins that represent another DNA-binding family. The Y-box binding proteins including dbpA (5), dbpB (5), EF1A (6) YB-1 (7), chkYB-1 (8), NSEP-1 (9), FRGY1 (10), FRGY2 (10) and YB-3 (11) contain a highly conserved DNA-binding domain known as the cold-shock domain (12,13). In spite of their very high degree of sequence similarity in the basic DNA-binding domain, a diverse range of functions has been described; some of them function as a positive or negative CCAAT-binding transcription factor (10,14,15), and others as a single-stranded DNA-binding protein (9,16), factor binding to depurinated DNA (17,18), and messenger ribonucleoprotein components (19–21). Little is known about the regulatory manner of expression, interaction with other factors, and their biological roles in the cell of this class of DNA-binding proteins.

During the screening of cDNAs encoding regulatory factors that interact with an element (site B) locating in the rat aldolase B promoter (22,23), we isolated a cDNA that encodes a novel member of the Y-box binding protein family, and designated it as RYB-a (Rat Y-box Binding protein-a). We describe here the tissue-specific distribution and developmental stage-specific expression of RYB-a mRNA. We also describe that RYB-a gene is an example of genes that are under control of growth signals.

MATERIALS AND METHODS

Screening the expression library

A λgt11 expression library was constructed from cDNA prepared by random priming of adult liver mRNA. An aliquot of the unamplified library (6×10^6) was screened directly according to the method described by Singh *et al* (24). Site B oligonucleotide, which was 5′ end-labeled with [γ-^{32}P]ATP by using polynucleotide kinase, was multimerized with T4 DNA ligase and used as a probe. The library was plated at a density of about 40,000 plaque forming units (pfu) per 143 cm^2 dish. The plates were incubated at 42°C for 4 h until plaques became visible, and were then overlaid with nitrocellulose filters impregnated with isopropyl thiogalactoside (IPTG) to induce cDNA expression. After 6 h at 37°C, the filters were blocked by soaking in a buffer (25 mM NaCl, 2 mM $MgCl_2$, 5 mM DTT, and 50 mM Tris–HCl, pH 7.9) containing 5% non-fat dry milk. The filters were incubated with the multimerized DNA probe (10^7 cpm/ml) for 6 h at 4°C in the binding buffer containing 1 mg/ml salmon sperm DNA. The filters were then washed 3 times for 15 min with the same buffer at 4°C and exposed to X-ray film. One clone (λ139) which contained a 0.4 kb *Eco*RI insert was obtained. Another clone, a 1.5 kb clone containing the entire protein-coding sequence of RYB-a cDNA (λ17), was isolated by screening of an oligo(dT)-primed λgt11 library from adult rat liver.

*To whom correspondence should be addressed

DNA sequencing

*Eco*RI fragments from the isolated cDNA clones were subcloned into pBluescript KS+ plasmid vector and sequenced either manually or using an ABI 373a automatic sequencer according to protocols from the manufacturer (Applied Biosystems). Homology searches were performed using FASTA program (25).

RNA and DNA analysis

Radiolabeled probes were prepared by the random primer labeling kit (TAKARA). For Northern blot analysis, total RNA was isolated according to LeMeur *et al.* (26). RNA was electrophoresed in a 1.2% formaldehyde gel. Ethidium bromide was added to each RNA sample to ensure that equivalent amounts of RNA were loaded onto the gel. After electrophoresis, RNA was transferred to a nylon membrane (Amersham). For Southern blot analysis, genomic DNA was prepared from rat liver according to the standard protocols (27). 10 μg of DNA was digested with restriction enzymes to completion, separated on a 0.9% agarose gel, and blotted onto a nylon membrane. The blots were hybridized overnight at 65°C with appropriate probes (1×10^6 cpm/ml). After hybridization, the filters were washed in 2× SSC containing 0.1% SDS at room temperature for 5 min, followed by two 15 min washes at 65°C in 0.1× SSC containing 0.1% SDS. Hybridization solutions contained 0.75 M NaCl, 50 mM Tris−HCl (pH 8.0), 2 mM EDTA, 1× Denhardt's reagent, and 50 mg/ml salmon sperm DNA. The filters were then autoradiographed for 7−72 h at −80°C with an intensifying screen.

Cell lines and cell culture

Mouse BALB/c 3T3 fibroblast cells (clone A31) were obtained from Japanese Cancer Research Resources Bank. New cultures were started every one month from frozen stock to prevent phenotypic changes of the cells. Cells were cultured in DME/F12 medium supplemented with 200 units/ml penicillin G, 0.1 mg/ml streptomycin sulfate, and 10% fetal calf serum (FCS) and incubated at 37°C in a humidified atmosphere of 5% CO_2. Quiescent cells (1×10^5 cells in 60 mm culture dish or 3×10^5 cells in 90 mm culture dish) were prepared by placing cells under serum-starved conditions (0.3% FCS for 72 h) after growth in the normal (FCS supplemented) medium. Quiescent cells were stimulated with 10% FCS for cell proliferation. To maintain the cells without adhesion, monolayer cells were detached with 0.125% trypsin/0.02% EDTA and cultured in dishes entirely coated with 2 mg/60 mm dish or 5 mg/90 mm dish of poly(2-hydroxyethylmethacrylate) [poly(HEMA)] (Aldrich Chemical Co.) (28).

RESULTS

Isolation of a cDNA encoding a protein with conserved DNA-binding motif

By screening a rat liver λgt11 expression library (24), we obtained a clone named λ139 that displays strong binding activity to the site B of the aldolase B promoter. This clone was used for further screening to obtain full-length cDNA and consequently two additional clones were isolated; one λ17, contains an insert of 1,520 bp long and ends with a poly (A) tail that is preceded by a consensus polyadenylation signal AATAAA at position 1,478 (Fig. 1). The nucleotide sequence (GGCGGCATGA) around the AUG codon at position 51 matches a consensus sequence (GCC-

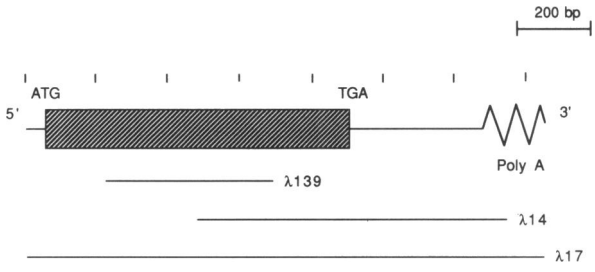

Figure 1. Schematic representation of RYB-a cDNA clones. The open reading frame is indicated by the striped box. Locations of the isolated overlapping cDNA clones are shown.

$^{A}/_{G}$CCATGG) for the translation initiation signal (29). An open reading frame indicates that this clone encodes a polypeptide of 291 amino acids with estimated molecular weight of 31,200 Da. A sequence homology search using the Genebank data with FASTA program revealed high sequence homologies with human DNA-binding protein A (dbpA) and chicken YB-1 (chkYB-1), to degrees of 76.4% and 68.0% for the mRNAs, and 71.0% and 52.4% for the proteins, respectively. DbpA binds to epidermal growth factor enhancer with unknown function (5) and chkYB-1 binds to the promoter region of the liver-specific, estrogen-dependent very-low-density apolipoprotein II gene (8). Since they are members of Y-box binding proteins that have common conserved DNA-binding domains (8), we designated this protein as RYB-a (Rat Y-box Binding protein-a). A sequence search for conserved motifs revealed that there are two stretches of basic amino acid from Arg 165 to Arg 177 and Arg 245 to Arg 259 (both regions have eight basic amino acid residues out of 13−15 amino acids), similar to the previously described nuclear translocation signals (14,30). A potential Ser residue at 33 for phosphorylation by MAP kinase (Pro−X−Ser/Thr−Pro) (31,32), four potential casein kinase II phosphorylation sites (Ser/Thr−X−X−Asp/Glu) (residues 103,125,149 and 288) (33) and three putative *N*-linked glycosylation sites (Asn−X−Tyr/Ser/Thr) (residues 93, 101 and 147) (34) are also detected. It might be worth noting that the sequence, ATTTA, found in many cytokines and proto-oncogene mRNAs, was found in 2 places at positions 1,372 and 1,397 in the 3'-noncoding region of the cDNA. This sequence is thought to be involved in the mRNA destabilization (35).

In Fig. 2, the amino acid sequence of RYB-a is aligned with four members of the Y-box binding protein family so as to give the highest homology. According to the sequence alignment, the RYB-a sequence can be divided into three domains, A, B and C. The domain B (residues 75−182) is the most conserved region. This domain seems to be responsible for the DNA-binding, considering from the fact that the core region of this domain (residues 95−114) displays high homology to the cold-shock domain (CSD) found in prokaryotic and eukaryotic DNA-binding proteins (12,13). Tafuri and Wolffe have shown that removal of the corresponding CSD region from FRGY1, a *Xenopus* homolog of YB-1, leads to a loss of specific DNA binding (15). The domain A (residues 1−74) is a relatively distinct region among the Y-box binding proteins. This region is characteristically rich in Pro (18.9%) and Ala (28.4%), which are known to constitute activation domains of a number of transcription factors (36, 37). The carboxyl-terminal domain C

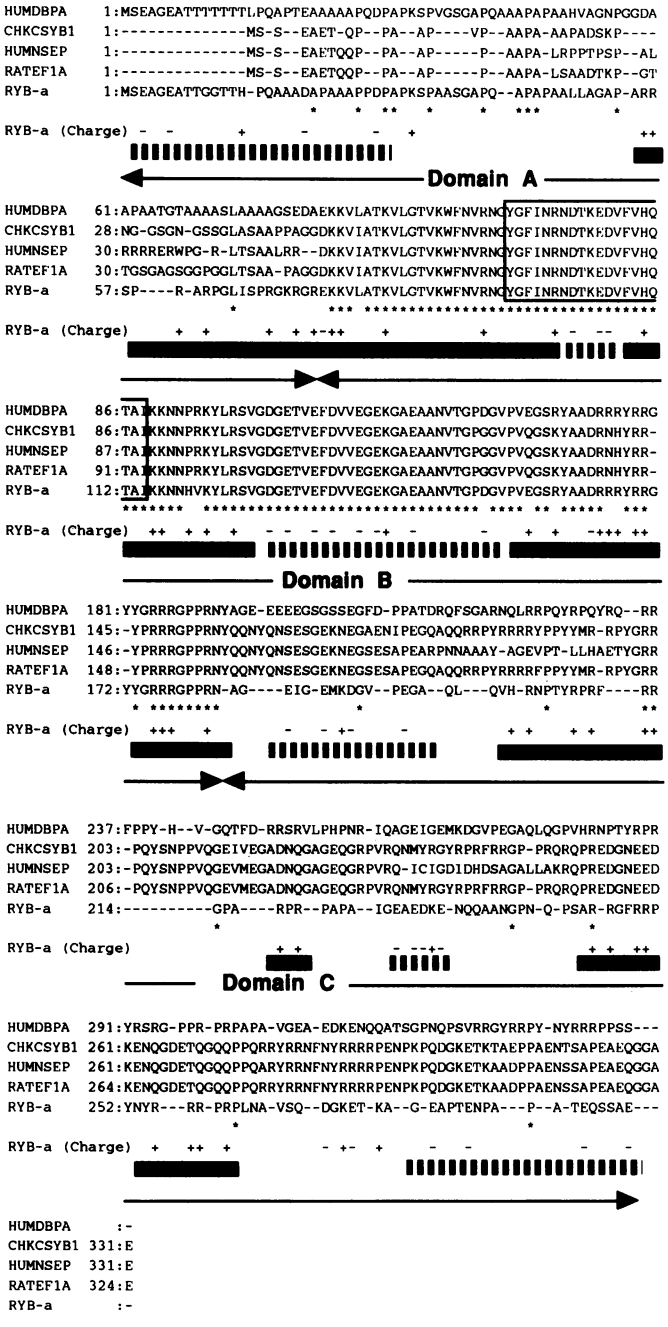

Figure 2. Amino acid sequence alignment of RYB-a with known members of the Y-box protein family. Amino acid sequences are aligned by GENETYX-Homology software system, version 2.2.0 (Software Development Co. Ltd). The accession numbers for these sequences that shown in the figure (top to bottom) are PS0014 (HUMDBPA), L13032 (CHKCSYB1), M83234 (HUMNSEP) and M95793 (RATEF1A) and D28557 (RYB-a). HUMDBPA, human DNA-binding protein A (dbpA) (5); CHKCSYB1, *Gallus fallus* YB-1 (8); HUMNSEP, human nuclease sensitive element binding protein (9); RATEF1A, rat enhancer factor 1 subunit A (6). Three putative domains, (domain A, B and C) are shown. Amino acids conserved among the members listed are indicated by asterisks. Consensus sequence for the cold-shock domain (CSD) is boxed. Alternating positively and negatively charged residue clusters are given by solid and dashed bars, respectively.

does not exhibit exact amino acid homology. One unusual aspect obtained by the inspection of the charged amino acid residues is an alternating pattern of positively and negatively charged regions (Fig.2). This pattern, so-called charge zipper domain (6,11,15), may be important for RYB-a activity through protein−protein multimerization.

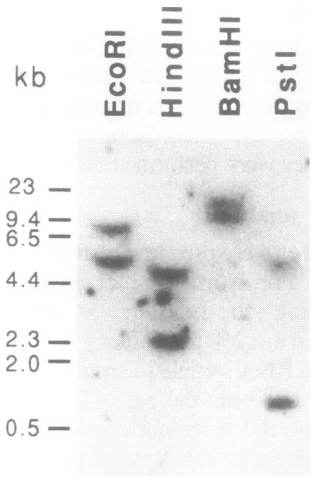

Figure 3. Southern blot analysis of rat liver genomic DNA (10 μg) digested with *Eco*RI, *Hin*dIII, *Bam*HI, and *Pst*I. The blot was hybridized to the 1.5 kb cDNA insert of RYB-a clone λ17. Migration of DNA standards (λ *Hin*dIII fragments) is given.

Genomic Southern blotting

To estimate copy numbers of the RYB-a gene, a high-stringency Southern blot hybridization was carried out. The result in Fig. 3 shows that the labeled probe hybridized with two DNA fragments from liver genomic DNA that was digested with restriction enzymes which have no cutting-site within the RYB-a cDNA sequence. Using a different probe corresponding to the 3′-noncoding region also gave the same two bands in each reaction (data not shown). Thus, it appears that RYB-a gene belongs to a dispersed gene family. Most probably the rat genome contains two RYB-a-related genes. In the mouse, four YB-1 related loci have been reported (7). It is possible that some of these loci are related to RYB-a gene.

Tissue-specific and developmental stage-specific expressions of RYB-a mRNA

Figure 4A shows the tissue-distribution of RYB-a mRNA. RYB-a mRNA was not detected in the liver and kidney. Low levels of expression were detected in the brain and heart, and high levels of the mRNA were detected in the skeletal muscle and spleen. Interestingly, there appears to be an inverse correlation with RYB-a mRNA levels and those of aldolase B mRNA. For example, the lowest levels of RYB-a mRNA are observed in the liver and kidney concurrently with the highest levels of aldolase B mRNA. Conversely, the highest RYB-a mRNA levels are seen in the skeletal muscle and spleen in which no aldolase B mRNA is detected. Fig. 4B shows developmental stage-specific expression of the RYB-a gene in the liver. At days 14 and 16, fetal livers accumulated high levels of RYB-a mRNA, but thereafter, the levels of the mRNA decreased drastically; in the new born and adult livers, the concentrations of RYB-a mRNA are significantly low as compared to those of day 14 and day16 fetal livers. Thus, mRNA levels of RYB-a and those of aldolase B showed complete inverse relationship in the liver during development.

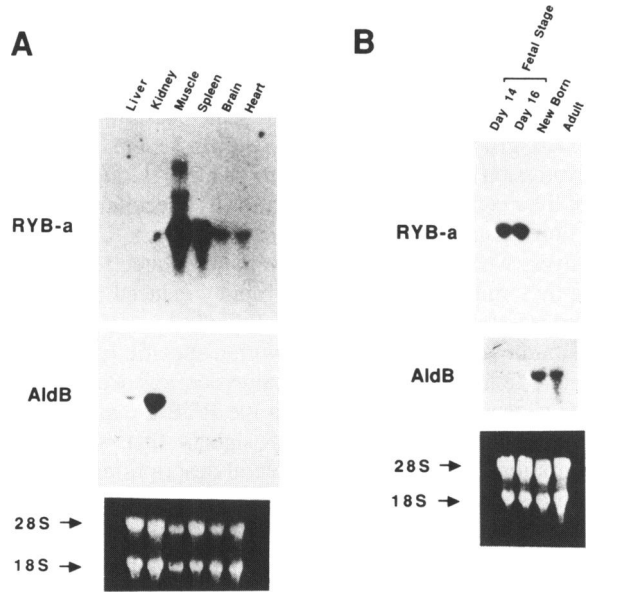

Figure 4. Northern blot analyses of the RYB-a mRNA. RNAs from various tissues (20 μg each) (**A**) and fetal livers (30 μg each) (**B**) were electrophoresed, blotted, and hybridized sequentially with 1.5 kb cDNA insert of RYB-a and aldolase B (AldB) cDNA (44). Ethidiumbromide staining of the gel in each experiment is shown to confirm equal loading.

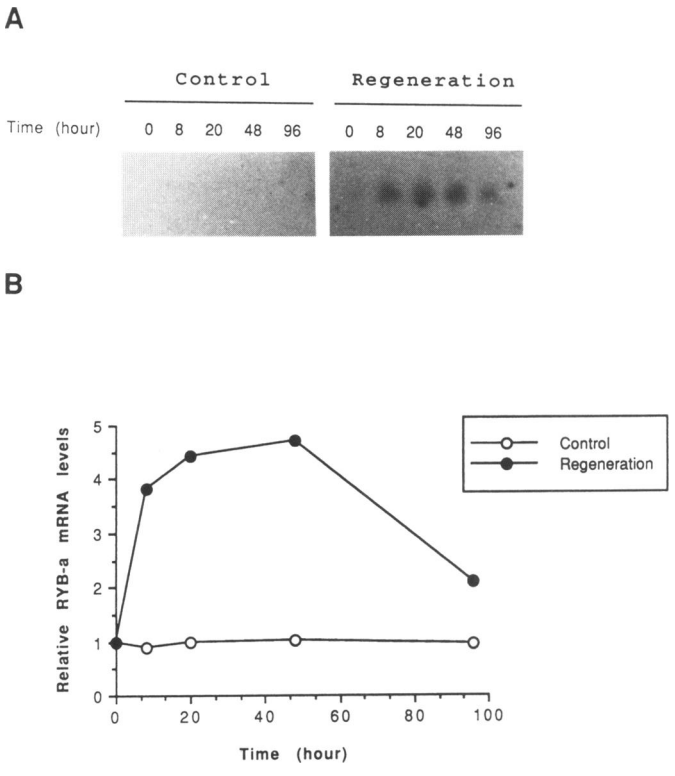

Figure 5. Expression of RYB-a mRNA in rat liver during regeneration. (**A**) 10 μg of total RNAs isolated at the indicated time from the sham-operated livers (control) and regenerating livers (regeneration) after partial hepatectomy (45) were examined for RYB-a expression by Northern blot analysis with the probe of 1.5 kb cDNA insert of RYB-a. (**B**) Relative RYB-a mRNA levels based on densitometric tracing of the autoradiogram in (A). The mRNA levels of control (open circle) and those of regeneration (solid circle) were expressed relative to that assayed in the normal adult rat liver. Experiments were performed in each point using two rat livers, one with hepatectomy and another with sham-operation.

Induction of RYB-a mRNA in regenerating liver

As mentioned above, RYB-a mRNA was not detectable in the adult liver while the mRNA was highly expressed in the fetal liver. This suggested that the expression of RYB-a mRNA in the liver relates to cell proliferation. To see this possibility, we examined whether or not the expression of RYB-a gene is induced during liver regeneration. Total RNAs isolated at different time periods after partial hepatectomy (regeneration) or sham operation (control) were blotted and probed with RYB-a cDNA. As shown in Fig. 5, the levels of RYB-a mRNA were elevated approximately 4-fold at 8 h and reached a maximal level 20−48 h in regeneration. However, the level of RYB-a mRNA in the control experiment did not change at all. This suggests that RYB-a mRNA is coordinately regulated with cell proliferation.

Growth-stimulated induction of RYB-a mRNA in fibroblast cells

The results obtained above prompted us to investigate in more detail whether the expression of RYB-a mRNA is correlated with cell proliferation. For this purpose, quiescent fibroblast BALB/c 3T3 cells were stimulated with serum and the levels of RYB-a mRNA were analyzed by Northern blotting. Entry into S phase was monitored by the expression of histone H2B gene (38). The mRNA level of ribosomal protein L35a was taken as an internal standard (39). Experimental time course is summarized in Fig. 6A. Quiescent cells expressed very low level of RYB-a mRNA, but the expression was detected 30 min after the stimulation (Fig. 6B, C). The expression gradually increased during G1 phase, and high level of the expression continues throughout the S phase (Fig. 6B). However, when the cells were blocked to proceed into S phase either by preventing cell adhesion (Fig. 6C) (40) or by treating with a specific tyrosine kinase inhibitor, genistein (Fig. 6D), the expression of RYB-a gene was almost completely suppressed. Genistein is known to inhibit the activities of tyrosine kinases such as the epidermal growth factor receptor and $pp60^{src}$, but scarcely inhibits the activity of serine and threonine kinases such as protein kinase A (41). These data thus suggest that a signal transduction cascade from tyrosine kinase is involved in the regulation of RYB-a gene expression.

DISCUSSION

In this paper, we described the analysis of a cDNA clone which encodes for a DNA-binding protein interacting with aldolase B promoter. The encoding protein termed as RYB-a (Rat Y-box Binding protein-a) contains the cold-shock domain (CSD) that has been found in many transcription factors including Y-box binding proteins (15). Since site B in the aldolase B promoter has close similarity with the Y-box (23), it is not surprising that Y-box related cDNA was obtained by the South-western method.

From the structural analysis of the cDNA, the primary translation product was shown to consist of 291 amino acids. Features of the putative RYB-a protein suggest potential mechanisms of the regulation. Domain A in amino-terminal region contains a Pro and Ala rich region that is thought to be

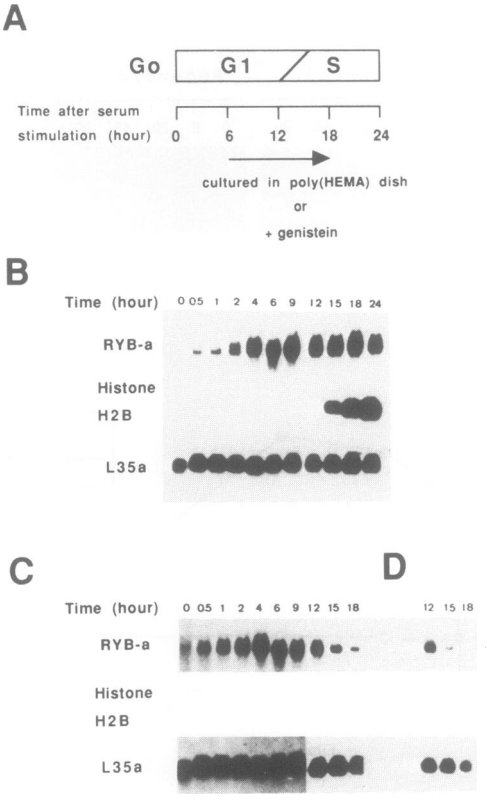

Figure 6. Induction of RYB-a gene expression by serum stimulation in mouse BALB/c 3T3 fibroblasts. (**A**) Schematic representation of the experimental system. Quiescent cells (Go) prepared by serum-starvation (0.3% FCS, 72 h) were stimulated with a medium supplemented with 10% FCS. To block the progression into S phase, cells were cultured either in a poly(HEMA)-coated dish or with 15 μg/ml of genistein for indicated time period. (**B**) Northern blot analyses of RYB-a gene expression in the control experiment. Judged from the induction of histone H2B gene, cells entered into S phase about 15 h after the stimulation. (C, D) Northern blot analyses of RYB-a gene expression in the absence of cell adhesion (**C**) and in the presence of genistein (**D**). 10 μg of total RNAs were hybridized sequentially with 1.5 kb cDNA insert of RYB-a, histone H2B cDNA (38) and L35a cDNA (39). The complete blockage for the cells to proceed into S phase was verified by no induction of histone H2B gene.

concerning with transcriptional regulation. The highly conserved domain (domain B) that contains CSD seemed to be responsible for DNA-binding. The putative charge zipper domain might mediate protein−protein interaction. The presence of casein kinase II and MAP kinase consensus regions suggests that RYB-a might be modulated by phosphorylation. Thus, in addition to the involvement of conserved DNA-binding region in domain B, interactions of the RYB-a protein with other proteins or/and DNA may be modulated by phosphorylation of the protein.

RYB-a gene is actively expressed in the skeletal muscle and the spleen of an adult rat, while other Y-box binding proteins such as YB-3 are expressed ubiquitously (11), suggesting diverse roles of the Y-box protein gene family. The expression pattern of RYB-a gene shows completely inverse relationship between aldolase B-expressing and non-expressing cells or tissues; the RYB-a mRNA level in the non-aldolase B-expressing livers at days 14 and 16 of fetal development is very high compared with those in new born and adult. Namely, RYB-a mRNA is expressed in the cells in which aldolase B gene is repressed. Since RYB-a mRNA level is high in rapidly developing tissues such as fetal liver at early stages, it seems that the expression of RYB-a gene is related to cell proliferation. Although the expression profile in several tissues such as the skeletal muscle and spleen argues against, the following results support the possibility that RYB-a gene is a growth-related, or a growth-inducible gene. Firstly, the expression of RYB-a mRNA was induced during regeneration of the liver cells after partial hepatectomy. Secondly, in BALB/c 3T3 fibroblasts, quiescent cells did not express RYB-a mRNA. However, when quiescent cells were stimulated to enter into S phase by serum, RYB-a gene was rapidly induced. Thirdly, once the progression into S phase was inhibited by detaching the cell adhesion or by treating the cells with a specific tyrosine kinase inhibitor, genistein, RYB-a gene expression was decreased. These results suggest the possibility that the RYB-a expression could be induced in association with signals that stimulate cell proliferation. There have been several descriptions that indicate proliferative response of the Y-box binding factors. Grant *et al.* (8) has reported that chkYB-1 expression was induced in the roosters liver treated with estrogen and in regenerating livers in rat. Since both treatments cause DNA replication, it seems that chkYB-1 expression is positively associated with DNA synthesis or cell proliferation. In addition, it has been shown that YB-1 mRNA level in helper T cells is induced by the stimulation of interleukin 2 that is known to push G1 cells for entering into S phase (42). Although there are no clear data addressing the molecular mechanisms involved in these inductions, our results using inhibitors of cell attachment and protein tyrosine kinase suggest the involvement of signal transduction cascade from cell adhesion or/and protein phosphorylation through tyrosine kinase in the expression of RYB-a mRNA.

Despite the high sequence homology of a putative DNA-binding domain containing CDS in the Y-box binding proteins, diverse functions have been reported. For example, the broad range of binding specificities of chkYB-1 has suggested that it could function in maintaining a potentially active chromatin configuration during DNA replication (8). On the other hand, recently Kashanchi *et al.* (43) reported that YB-1 could recognize Y-box-related sequences in the promoter of the human T-cell lymphotropic virus type I (HTLV-I) and the human immunodeficiency virus (HIV) LTR and transactivate their basal transcriptions. In the case of RYB-a, it is worth noting that the amino-acid sequences in amino-terminal and carboxyl-terminal regions have been shown to be relatively different from other Y-box binding protein family. Especially, the existence of a Pro and Ala rich region that is known to constitute a transcriptional regulatory domain in the amino-terminal region of RYB-a suggests its potential function as a transcription factor.

Considering the expression profile of aldolase B gene, it might be that RYB-a could function as a negative regulatory factor in the expression of aldolase B gene. In the fetal livers at days 14 and 16 of gestation, factor AlF-B which acts positively on site B (22,23) has, although to a lesser extent, already accumulated. If RYB-a acts negatively on aldolase B gene transcription, it would be interesting to know how RYB-a interferes with the positive action of AlF-B. Of much interest might be the protein−protein interaction through the putative charge zipper domain. This domain might regulate RYB-a activity by promoting or repressing protein−protein multimerization.

In any case, the remarkable conservation of the putative DNA-binding domain of RYB-a suggests the potential importance of this gene in the gene regulation of eukaryotic cells, particularly those undergoing cell proliferation.

ACKNOWLEDGEMENTS

We thank Prof. M.Muramatsu (Saitama Medical School) for his encouragement and valuable discussions. We also thank Dr M.Sato for helpful discussions in computer analysis and Mrs M.Seki for secretarial assistance. This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan, and The Asahi Glass Foundation.

REFERENCES

1. Pabo, C.O. and Sauer, R.T. (1984) *Annu. Rev. Biochem.*, **53**, 293–321.
2. Scott, M.P., Tamkin, J.W. and Hartzell, G.W. (1989) *Biochim. Biophys. Acta*, **989**, 25–48.
3. Hayashi, S. and Scott, M.P. (1990) *Cell*, **63**, 883–894.
4. Miller, J., McLachlan, A.D. and Klug, A. (1985) *EMBO J.*, **4**, 1609–1614.
5. Sakura, H., Maekawa, T., Imamoto, F., Yasuda, K. and Ishii, S. (1988) *Gene*, **73**, 499–507.
6. Ozer, J., Faber, M., Chalkey, R. and Sealy, L. (1990) *J. Biol. Chem.*, **265**, 22143–22152.
7. Spitkovsky, D.D., Royer-Pokora, B., Delius, H., Kisseljov, F., Jenkins, N.A., Gilbert, D.J., Copeland, N.G. and Royer,H.-D. (1992) *Nucleic Acids Res.*, **20**, 797–803.
8. Grant, C.E. and Deeley, R.G. (1993) *Mol. Cell. Biol.*, **13**, 4186–4196.
9. Kolluri, R., Torrey, T.A. and Kinniburgh, A.J. (1992) *Nucleic Acids Res.*, **20**, 111–116.
10. Tafuri, S.R. and Wolffe, A.P. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 9028–9032.
11. Cohen, I. and Reynolds W.F. (1991) *Nucleic Acids Res.*, **19**, 4753–4759.
12. Doniger, J., Landsman, D., Gonda, M.A. and Wistow, G. (1992) *New Biol.*, **4**, 389–395.
13. Wistow, G. (1990) *Nature*, **344**, 823–824.
14. Didier, D.K., Schiffenbauer, J., Woulfe, S.L., Zacheis, M. and Schwarz, B.D. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 7322–7326.
15. Tafuri, S.R. and Wolffe, A.P. (1992) *New Biol.*, **4**, 349–359.
16. Kolluri, R., Torrey, T.A. and Kinniburgh, A.J. (1992) *Nucleic Acids Res.*, **20**, 111–116.
17. Hasegawa, S.L., Doetsch, P.W., Hamilton, K.K., Martin, A.M., Okenquist, S.A., Lenz, J. and Boss, J.M. (1991) *Nucleic Acids Res.*, **19**, 4915–4920.
18. Lenz, J., Okenquist, S.A., Losardo, J.E., Hamilton, K.K.and Doetsch, P.W. (1990) *Proc. Natl. Acad. Sci. USA*, **88**, 3396–3400.
19. Deschamps, S., Viel, A., Garrigos, M., Denis, H. and le Maire, M. (1992) *J. Biol. Chem.*, **267**, 13799–13802.
20. Tafuri, S.R., Familari, M. and Wolffe, A.P. (1993) *J. Biol. Chem.*, **268**, 12213–1220.
21. Ranjan, M., Tafuri, S.R. and Wolffe A.P. (1993) *Genes Dev.*, **7**. 1725–1736.
22. Tsutsumi, K.,Ito, K. and Ishikawa, K. (1989) *Mol. Cell. Biol.*, **9**,4293–4931.
23. Tsutsumi, K., Ito,K., Yabuki, T. and Ishikawa, K. (1993) *FEBS Lett.*, **321**, 51–54.
24. Singh, H., LeBowitz, J.H., Baldwin, A.S. and Sharp, P.A. (1988) *Cell*, **52**, 415–423.
25. Pearson, W.R. and Lipman, D.J. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 2444–2448.
26. LeMeur, M., Glanville, N., Mandel, J.L., Gerlinger, P., Palmiter, R. and Chambon, P.(1981) *Cell*, **23**, 561–571.
27. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor University Press, Cold Spring Harbor.
28. Folkman, J. and Moscona, A. (1978) *Nature*, **273**, 345–349.
29. Kozak, M. (1987) *J. Mol. Biol.*, **196**, 947–950.
30. Dingwall, C. and Laskey, R.A. (1986) *Annu. Rev. Cell Biol.*, **2**, 367–390.
31. Clark-Lewis, I., Sanghera, J.S. and Pelech, S.L. (1991) *J. Biol. Chem.*, **266**, 15180–15184.
32. Gonzalez, F., Raden, D. and Davis, R. (1991) *J. Biol.Chem.*, **266**, 22159–22163.
33. Pinna, L.A. (1990) *Biochim. Biophys. Acta*, **1054**, 267–284.
34. Kornfeld, R. and Kornfeld, S. (1985) *Annu.Rev.Biochem.*, **54**, 631–664.
35. Sachs, A.B. (1993) *Cell*, **74**, 413–421.
36. Landschultz, W.H., Johnson, P.F. and McKnight, S.L. (1988) *Science*, **240**, 1759–1764.
37. Han, P., Brown, R. and Barsoum, J. (1991) *Nucleic Acids Res.*, 19, 7225–7229.
38. LaBella, F., Sive, H.L., Roeder, R.G. and Heintz, N. (1988) *Genes Dev.*, **2**, 32–39.
39. Tanaka, T., Wakasugi, K., Kuwano, Y., Ishikawa, K. and Ogata, K. (1986) *Eur. J. Biochem.*, **154**, 523–527.
40. Guadagno, T. and Assoian, R. (1991) *J.Cell Biol.*, **155**, 1419–1425.
41. Akiyama, T., Ishida, J.Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. and Fukami, Y. (1987) *J. Biol. Chem.*, **262**, 5592–5595.
42. Sabath, D.E., Podolin, P.L., Comber, P.G. and Prystowsky, M.B. (1990) *J. Biol. Chem.*, **265**, 12671–12678.
43. Kashanchi, F., Duvall, J.F., Dittmer, J., Mireskandari, A., Reid, R.L., Gitlin, S.D. and Brady, J.N. (1994) *J. Virol.*, **68**, 561–565.
44. Tsutsumi, K., Mukai, T., Tsutsumi, R., Mori, M., Daimon, M., Tanaka, T., Yatsuki, H., Hori, K. and Ishikawa, K. (1984) *J. Biol. Chem.*, **259**, 14572–14575.
45. Grisham, J.W. (1962) *Cancer Res.*, **22**, 842–849.