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Marked increases of two kinds of two-exon-skipped albumin mRNAs with aging and their further increase by treatment with 3′-methyl-4-dimethylaminoazobenzene in Nagase analbuminemic rats

(carcinogen/RNA splicing/mutation/protein secretion)

TAKASHI KANEKO*, HIROSHI SHIMA*, HIROYASU ESUMI†, MASAKO OCHIAI*, SUMI NAGASE‡, TAKASHI SUGIMURA*, AND MINAKO NAGAO*§

*Carcinogenesis Division and †Biochemistry Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo, Japan; ‡Department of Chemistry, Sasaki Institute, 2-2, Kandasurugadai, Chiyoda-ku, Tokyo, Japan

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ABSTRACT **Nagase analbuminemic rats (NARs) have a 7-base-pair deletion at the 5′ splice site of the HI intron of the albumin gene. The level of immunohistochemically albumin-positive hepatocytes is about 1 per 10^5 cells in neonatal NARs, increases with age, and further increases with chronic oral treatment with 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB). The mechanisms involved in the increase in albumin-positive hepatocytes during aging of NARs and their treatment with 3′-MeDAB were analyzed. NARs were found to have four species of albumin mRNA: intact mRNA and those lacking the regions corresponding to exon H, exon G–H, and exon H–I. In 4-week-old NARs, the level of intact albumin mRNA was about 1/4000 of that in normal rats and mRNA lacking the exon H sequence was the major species. In aged and 3′-MeDAB-treated aged NARs, all four species of mRNA increased and the relative proportion of mRNAs lacking two exon sequences to mRNAs lacking one exon sequence was greatly increased, suggesting that aging is associated with changes of the splicing pattern and that 3′-MeDAB treatment enhanced these changes. In aged NARs and 3′-MeDAB-treated aged NARs, there was an increase in the amount of aberrant 60-kDa albumin. The 60-kDa protein could be a translation product of mRNAs lacking two exons, the amount of which increases in aged NARs and 3′-MeDAB-treated NARs.**

Nagase analbuminemic rats (NARs) were established from a stock of Sprague–Dawley (SD) rats (1). The level of serum albumin in NARs is about 3000 times less than that of SD rats (2). This trait is transmitted in an autosomal recessive manner and has been shown to be associated with a 7-base-pair deletion starting from nucleotide (nt) 5 at the 5′ splice site of the H–I intron of the albumin gene (3). NARs are homozygous for this mutation. This mutation seems to be the cause of impaired albumin mRNA splicing in NARs, in which the level of cytoplasmic albumin mRNA is 100–400 times less than in SD rats, and the molecular sizes of nuclear albumin mRNAs are much larger than normal (4). Later studies have shown that in NARs most albumin mRNAs lack the exon H sequence and that the level of intact albumin mRNA is only 1% of that of total cytoplasmic albumin mRNAs (5, 6). Moreover, by analysis of a cDNA library constructed from NARs, we found an albumin mRNA lacking the exon G and exon H sequences (unpublished data).

Immunohistological analysis showed that 0.02% of the hepatocytes in NAR liver were albumin-positive 4 weeks after birth and that these cells increased with age to about 0.6% of the total at 20 weeks and also increased to several percent of the total during treatment with hepatocarcinogens, such as 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB) and 2-acetylaminofluorene (7). Hepatocarcinogens in foods, such as 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline and 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole, also induced albumin-positive cells (unpublished data), but *N*-nitrosodiethylamine did not, even though it is a hepatocarcinogen in rats (7).

In this study, we analyzed changes of albumin mRNAs in aged and 3′-MeDAB-treated aged NARs in which albumin-positive hepatocytes detected immunohistologically were increased.

MATERIALS AND METHODS

Experimental Animals. NARs were bred at Sasaki Institute, Tokyo, and fed a basal CE-2 diet (CLEA Japan, Tokyo). A diet containing 0.06% 3′-MeDAB was given to two male NARs from week 7 after birth for 18 weeks and four female NARs from week 7 after birth for 20–31 weeks, when the animals were sacrificed. Male SD rats (Charles River Japan, Kanagawa, Japan) of 9 weeks old were used as controls.

Oligonucleotide Probes and Primers. Probes and primers of the albumin gene were synthesized in a model 381A DNA synthesizer and purified on an OPC column (Applied Biosystems). The primers and probes used were as follows: G981, CTCAATAGCTGCTGACTTTG [sense strand of exon G corresponding to nt 981–1000 of albumin cDNA (8)]; F771, ATTGGCAACAGAGGTTACCA [sense strand of exon F corresponding to nt 771–790 of albumin cDNA (8)]; H5S, GTTTTTGTATGAATATTCAA (sense strand of the 5′ end of exon H); H3A, CACTGTGCCGTAGCAGGCAG (antisense strand of the 3′ end of exon H); E3S, GAGAGAGCCTTCAAAGCCTG (sense strand of the 3′ end of exon E); L5A, TCACCAGCTCAGCGAGAGCC (antisense strand of the 5′ end of exon L); 20GJ, AACCAGAATGGTGCCCAGGA (conjugate of 10 nt of the antisense strand of the 3′ end of exon G and 10 nt of the 5′ end of exon J); 30FI, CTGAAATTCTGCAAGCCTGTCATCCGCGCA (conjugate of 15 nt of the antisense strand of the 3′ end of exon F and 15 nt of the 5′ end of exon I); 30GI, CTGAAATTCTGCAAGGTGCCCAGGAAGACA (conjugate of 15 nt of the antisense strand of the 3′ end of exon G and 15 nt of the 5′ end of exon I).

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Abbreviations: NAR, Nagase analbuminemic rat; 3′-MeDAB, 3′-methyl-4-dimethylaminoazobenzene; SD, Sprague–Dawley; nt, nucleotide(s).

§To whom reprint requests should be addressed.

Isolations of RNA and Poly(A)$^+$ RNA. Total RNA was isolated from the liver by the acid guanidinium isothiocyanate/phenol/chloroform extraction method (9). Poly(A)$^+$ RNA was isolated using Oligotex-dT30 (Takara Shuzo, Kyoto).

Northern Blot Analysis. Samples were separated by electrophoresis in a formaldehyde/agarose gel, blotted onto nitrocellulose filters, and hybridized with labeled probes. Probes of exon H, which was obtained by PCR with H5S and H3A primers, of rat albumin cDNA 9-1, which contains all exons from exon F to exon N except exon H (unpublished data), and of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, provided by P. Jeanteur (10), were labeled with a multiprime labeling system (Amersham) and [α-^{32}P]dCTP. The other probes were labeled at the 5′ end with [γ-^{32}P]ATP and T4 polynucleotide kinase (11).

cDNA Synthesis, Amplification by PCR, and Sequence Analysis. The first strand of cDNA was synthesized from poly(A)$^+$ RNA obtained from the liver of a 3′-MeDAB-treated male NAR of 25 weeks old (12). The cDNAs were separated by electrophoresis and those of 2050–2300 nt, which were similar in size to normal albumin mRNA, were eluted. With this eluate as template, a region of the albumin cDNA containing exon E through exon L was amplified by PCR with E3S and L5A as primers (see Fig. 2). These PCR products were separated by electrophoresis in 1% NuSieve agarose (FMC) containing ethidium bromide (0.2 μg/ml). The three main bands were eluted separately. The longest fragment (expected not to contain exon H) was digested with *Pst* I and *Hin*dIII, which have sites in exon G and exon I sequences, respectively, and the digests obtained were ligated to these sites in Bluescript SK(+) (Stratagene) to obtain plasmid −H (see Fig. 2). The intermediate-sized fragment (expected not to have exon H and exon I sequences) was digested with *Pst* I and *Sal* I, which have sites in exon G and exon J sequences, respectively, and the digests obtained were ligated to these sites in the Bluescript to yield plasmid −HI. The shortest fragment (expected not to have exon G and exon H sequences) was digested with *Hin*dIII and *Alu* I, which have sites in exon I and exon F sequences, respectively, and the digests obtained were ligated into the *Hin*dIII and *Hin*cII sites of the Bluescript to yield plasmid −GH. The sequences were analyzed with Sequenase (United States Biochemical). The sequence primers used were G981, G981, and F771 for plasmid −H, plasmid −HI, and plasmid −GH, respectively.

RNA Probe Mapping. After *Bam*HI digestion of plasmid −GH, plasmid −H, and plasmid −HI, RNA probes were made with a TransProbe T kit (Pharmacia) using an appropriate RNA polymerase, T3 or T7. A sample of 50 μg of total RNA and 100,000 cpm of probe was dissolved in 30 μl of 80% (vol/vol) formamide/0.4 M NaCl/0.05 M Pipes·NaOH, pH 6.4/1 mM EDTA, heated at 80°C for 5 min, and then kept at 42°C for 3 nights. Then 350 μl of 10 mM Tris·HCl, pH 7.5/5 mM EDTA/300 mM NaCl was added and the samples were digested with RNase A (10 μg/ml) and RNase T1 (0.5 μg/ml) at 30°C for 30 min. After treatments with proteinase K and SDS, samples were extracted with phenol/chloroform, precipitated with ethanol, and subjected to electrophoresis in a 6% polyacrylamide gel containing 8 M urea (11). About 15 μg of yeast tRNA was used as a negative control.

Immunoprecipitation and Western Blot Analysis. A cytosol fraction and a microsome fraction were obtained from a liver homogenate (13). Protein samples of 5 mg were incubated with 160 μg of rabbit anti-rat albumin IgG (Organon Teknika–Cappel) at 4°C overnight and then precipitated with 20 μl of protein A-Sepharose (Pharmacia) (14). Materials were separated by electrophoresis (15) and transferred to a membrane filter, which was incubated sequentially with a goat anti-rat albumin IgG (40 μg/ml) (Organon Teknika–Cappel) and biotinylated rabbit anti-goat IgG (heavy and light chain) immunoglobulin (Vector Laboratories). A Vectastain ABC kit (Vector Laboratories) was used for staining.

RESULTS

Expression of the Exon-H-Skipped mRNA. Titration blots of SD rat liver poly(A)$^+$ RNA revealed that 8 μg of RNA from a 4-week-old NAR and 0.02 μg of RNA from a 9-week-old SD rat gave almost the same intensities of hybridization bands with the albumin cDNA probe (Fig. 1*A*, lanes 1 and 4) in accordance with a previous finding on cytoplasmic albumin mRNA (4). Since the amount of albumin mRNA in the poly(A)$^+$ RNA fraction of normal rats of 4–9 weeks old was constant (16), an age factor was probably not involved in this 400-fold difference between the NARs and SD rats. mRNAs of a 31-week-old NAR gave a broad band on Northern blot analysis (Fig. 1*A*, lane 2) and seemed to be composed of multiple species of albumin mRNAs of nearly normal and smaller sizes. These heterogeneous species of albumin mRNAs were increased in a male NAR of 25 weeks old that had been treated with 3′-MeDAB for 18 weeks (Fig. 1*A*, lane 3), whereas the level of GAPDH mRNA, an internal marker, remained constant (Fig. 1*B*). Densitometric analysis (Shimadzu CS-9000) revealed that the total heterogeneous albumin mRNAs detected with a cDNA probe were increased 6-fold and 16-fold in aged and 3′-MeDAB-treated aged NARs, respectively.

With the exon H probe, normal-sized mRNA was detected in a 4-week-old NAR, but its level was about 4000 times less than that in a 9-week-old SD rat (Fig. 1*C*, lanes 1 and 4). The results of Northern blot analyses using the albumin cDNA and exon H probes imply that most of the albumin mRNA in NARs detected with cDNA probe did not contain the exon H sequence and that in the 4-week-old NAR only about 10% of the mRNA, which was probably intact mRNA, contained the exon H sequence. The level of mRNA hybridized with the exon H probe increased 2-fold and 3-fold in 31-week-old and 3′-MeDAB-treated 25-week-old NARs, respectively, in contrast to 6-fold and 16-fold increase of those hybridized with the cDNA probe, as mentioned above. Thus normal mRNA seems to constitute about 3% and 2% of spliced albumin mRNA, in aged and 3′-MeDAB-treated aged NARs, respectively. The exon H probe hybridized more strongly with larger-sized mRNAs of aged and 3′-MeDAB-treated aged NARs (Fig. 1*C*, lanes 2 and 3) than in that of SD rats (Fig. 1*C*, lane 4), indicating a low efficiency of exon H splicing in

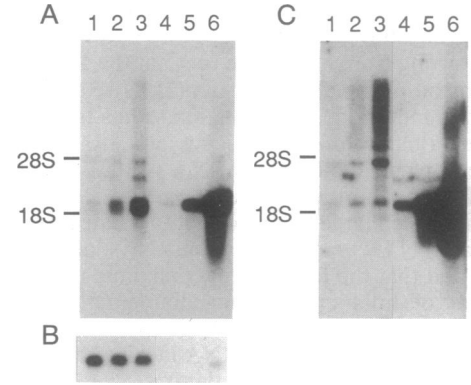

FIG. 1. Northern blot analysis of albumin mRNA of NAR and SD rats. The filter was hybridized sequentially to the albumin cDNA probe (*A*), GAPDH cDNA probe (*B*), and exon H probe (*C*). Samples of 8 μg of poly(A)$^+$ RNAs from a 4-week-old male NAR (lane 1), a 31-week-old male NAR (lane 2), a 25-week-old 3′-MeDAB-treated male NAR (lane 3), and 0.02 μg (lane 4), 0.2 μg (lane 5), and 2 μg (lane 6) of poly(A)$^+$ RNA from a 9-week-old male SD rat are shown.

NARs. 3′-MeDAB treatment increased the levels of these species of mRNAs.

Characterization of Albumin mRNA Lacking Exon H. To characterize the shorter albumin mRNAs that do not contain the exon H sequence, we amplified albumin cDNA of a 3′-MeDAB-treated aged male NAR by the PCR with the E3S and L5A primers. On electrophoresis, three different-sized fragments were detected as major products between the 603- and 872-base-pair markers (Fig. 2). In this PCR, if intact albumin mRNA is present, it should be detected as a 979-nt band, but the three main bands detected were shorter than 979 nt. Since only about 2% of the mRNA contains the exon H sequence in 3′-MeDAB-treated NARs, intact albumin might not be visualized with ethidium bromide even after PCR amplification. From the sizes of the bands, we assumed that the longest sequence (846 nt) did not contain the exon H, the second longest (748 nt) did not contain the exon H–I sequence, and the shortest (631 nt) did not contain the exon G–H sequence (see Fig. 2). DNA fragments eluted from the gel were digested with restriction endonucleases, ligated into Bluescript, and named plasmid −H, plasmid −GH, and plasmid −HI, respectively, as illustrated in Fig. 2. Sequence analysis of these three cDNA clones showed that splicing took place precisely between exons G and I, exons F and I, and exons G and J, respectively (data not shown).

Presence of Three Aberrant mRNAs in Young, Aged, and 3′-MeDAB-Treated Aged NARs. The existence of three species of exon-H-skipped mRNAs in young, aged, and 3′-MeDAB-treated aged NARs was confirmed by RNA probe mapping. With RNA probe prepared from plasmid −H, the 233-nt fragment, which should be protected by the mRNA containing exon G joined to exon I (−H mRNA) (see Fig. 2), was detected in a 4-week-old NAR (Fig. 3*A*, lane 2) as well as in mRNAs from an aged and a 3′-MeDAB-treated aged NAR (lanes 3 and 4). The 161-nt fragment should reflect mRNAs having the exon G sequence not joined to the exon I sequence (see Fig. 2). The 72-nt fragment should reflect mRNAs having the exon I sequence not joined to the exon G sequence. When the probe was hybridized with yeast tRNA, no band was observed.

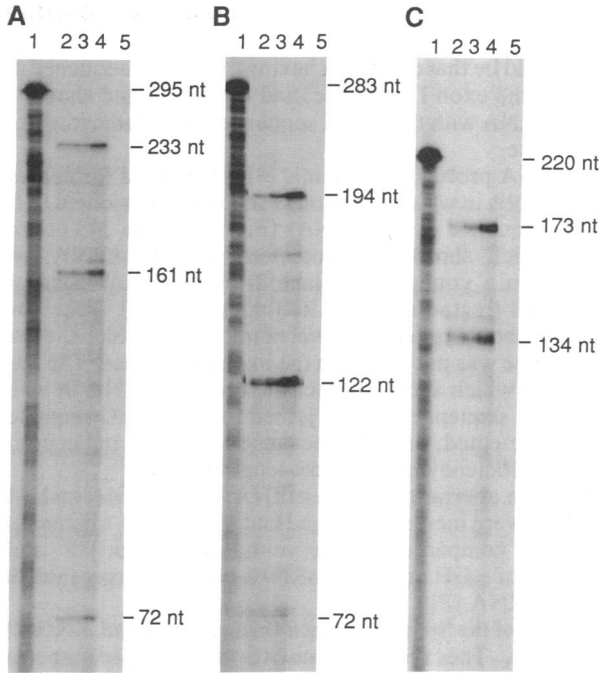

FIG. 3. RNA probe mapping of albumin mRNA of young, untreated aged, and 3′-MeDAB-treated aged NARs. RNA probes were prepared from plasmid −H (*A*), plasmid −GH (*B*), and plasmid −HI (*C*). These RNA probes were made from plasmids digested with *Bam*HI. Lanes: 1, 100,000 cpm of RNA probe; 2, 50 μg of total RNA from a 4-week-old male NAR; 3, 50 μg of total RNA from a 31-week-old male NAR; 4, 50 μg of total RNA from a 3′-MeDAB-treated 25-week-old male NAR; 5, 15 μg of yeast tRNA. Samples were annealed with the probe (100,000 cpm), digested with RNase, and subjected to electrophoresis.

The RNA probe from plasmid −GH was used for analysis of mRNA having the exon F sequence joined to the exon I sequence (−GH mRNA) (Fig. 3*B*). The 194-nt fragment, which should be protected by −GH mRNA, was detected in

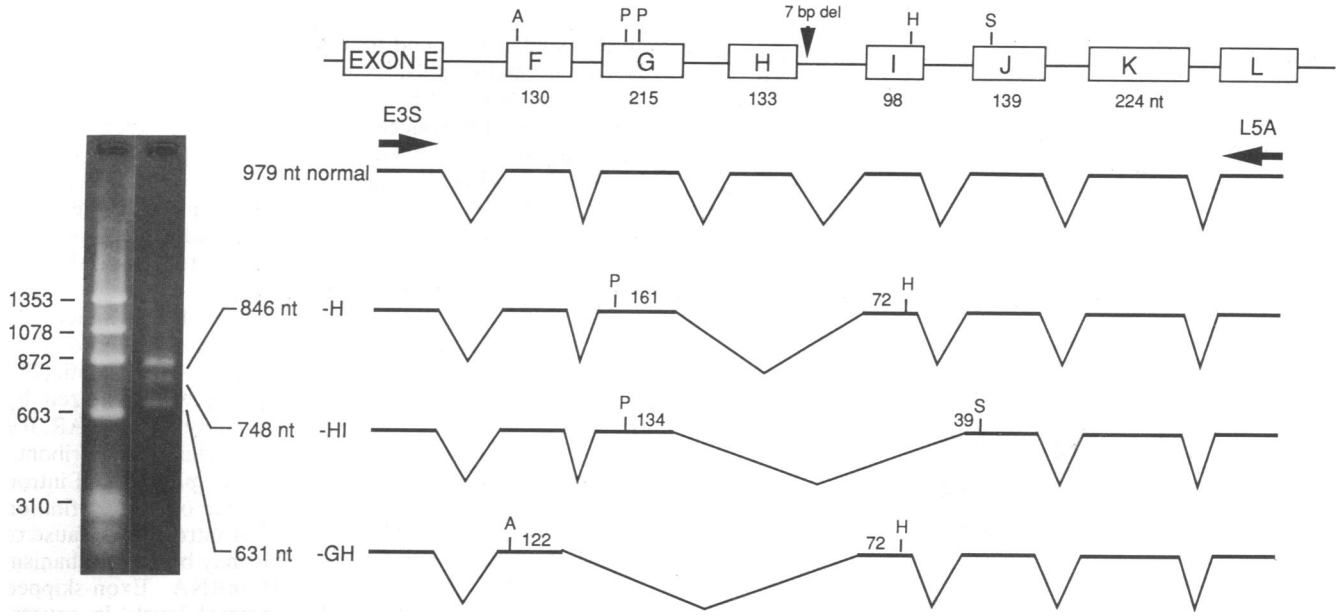

FIG. 2. Size estimation of PCR-amplified products. cDNA was synthesized from poly(A)$^+$ RNA of a 3′-MeDAB-treated male NAR, and after electrophoresis, cDNAs of about normal-sized albumin mRNA were eluted. The eluted cDNAs were PCR-amplified with the E3S and L5A primers and separated by electrophoresis (right lane). The left lane shows the positions of size markers (in nt). Of exons Z and A to N present in the albumin gene, only exons E to L are shown. A, *Alu* I; P, *Pst* I; H, *Hin*dIII; S, *Sal* I. Each fragment was cut with the restriction endonucleases and ligated into Bluescript.

a young NAR (lane 2), as well as in aged and 3′-MeDAB-treated aged NARs (lanes 3 and 4, respectively). The 122-nt band should be that of mRNA having the exon F sequence not joined to the exon I sequence, and the 72-nt band should be that of mRNA with the exon I sequence not joined to the exon F sequence.

The RNA probe from plasmid −HI was used for analysis of the mRNA in which the exon G sequence was joined to the exon J sequence (−HI mRNA) (Fig. 3*C*). The 173-nt fragment, which should be protected by −HI mRNA, was detected in a young NAR (lane 2) as well as in aged and 3′-MeDAB-treated aged NARs (lanes 3 and 4). The 134-nt fragment should reflect the level of mRNAs in which the exon G sequence was not joined to the exon J sequence. The 39-nt fragment, which should reflect the level of mRNA in which the exon J sequence was not joined to the exon G sequence, was not detected, probably because of the low radioactivity and low efficiency of the short-length probe.

All three aberrant albumin mRNAs (−H, −GH, and −HI mRNAs) were increased in aged and 3′-MeDAB-treated aged NARs as compared with a young NAR. Further, these increases of −GH and −HI mRNAs were far larger than that of −H mRNA (Fig. 3).

Change of the Splicing Pattern During Aging and 3′-MeDAB Treatment. The relative amounts of the three aberrant mRNAs were analyzed on Northern blots. Poly(A)$^+$ RNAs obtained from young, aged, and 3′-MeDAB-treated aged NARs were separated by electrophoresis for 20 hr. On hybridization with the albumin cDNA probe, three distinct bands were observed (Fig. 4). Northern blot analyses with the synthetic oligonucleotide probes 30GI, 20GJ, and 30FI indicated that the biggest mRNA was −H mRNA, the intermediate sized mRNA was −HI mRNA, and the smallest mRNA was −GH mRNA (data not shown). In a 4-week-old NAR, the major species was −H mRNA but, in a 31-week-old NAR, the proportions of −GH and −HI mRNAs were increased, although their levels were less than that of −H mRNA. In a 3′-MeDAB-treated 25-week-old NAR, the levels of the three mRNAs were almost the same. These results indicate that the splicing pattern changed in aged NAR and that this change was enhanced by 3′-MeDAB treatment.

A similar tendency was observed in another experiment using different NARs from those described above: A 4-week-old male gave a pattern similar to that in Fig. 4*A*; 43-, 51-, and 60-week-old males gave patterns similar to that in Fig. 4*B*; and a 3′-MeDAB-treated 25-week-old male and four 3′-MeDAB-treated females of 27–38 weeks old gave patterns similar to that in Fig. 4*C* (data not shown).

Increase of Smaller-Sized Aberrant Species of Albumin. Deletion of the exon H sequence results in the loss of 133 bases in the coding region and premature termination of translation in the exon I sequence by a frameshift, and the expected size of the protein coded by −H mRNA was 41 kDa.

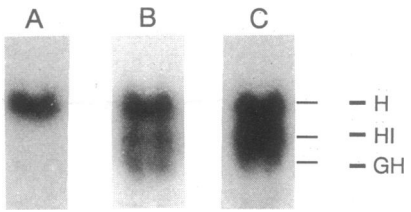

FIG. 4. Change of the splicing pattern in aged and 3′-MeDAB-treated aged NAR by Northern blot analysis with an albumin cDNA probe. A blot after longer electrophoresis was hybridized to a ^{32}P-labeled albumin cDNA probe. The exposure time was changed appropriately to obtain the same signal intensities of the top band of −H mRNA in lanes A–C. Lanes: A, 4-week-old male NAR mRNA; B, 31-week-old male NAR mRNA; C, 3′-MeDAB-treated 25-week-old male NAR mRNA.

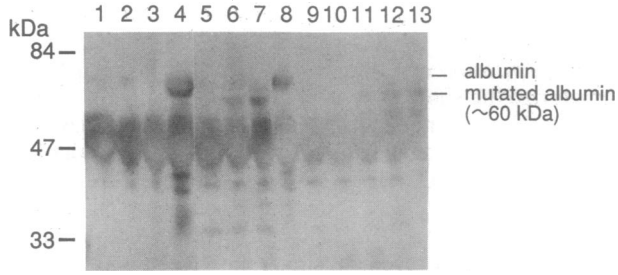

FIG. 5. Western blot analysis of albumin of young, aged, and 3′-MeDAB-treated aged NARs. Immunoprecipitated albumin from 5 mg of protein of each fraction was subjected to Western blot analysis using anti-rat albumin IgG. Lanes: 1–4, cytosolic fractions; 5–13, microsomal fractions; 1 and 5, 4-week-old male NARs; 2 and 6, 31-week-old male NARs; 3 and 7, 3′-MeDAB-treated 25-week-old male NARs; 4 and 8, 9-week-old male SD rats; 9 and 10, 4-week-old female NARs; 11–13, 3′-MeDAB-treated female NARs of 27–28 weeks old.

The exon H–I sequence deletion, a 231-nt loss, and the exon G–H sequence deletion, a 348-nt loss, are in frame. Therefore, the −HI and −GH mRNAs were expected to encode 60-kDa and 56-kDa aberrant albumins, respectively. On Western blot analysis of immunoprecipitates, albumin was detected in both the cytosolic and microsomal fractions of normal rats (Fig. 5). In NARs, a small amount of normal-sized 67-kDa albumin was detected in the cytosolic fraction, but its amount did not change significantly in aging or 3′-MeDAB treatment (lanes 1–3). In the microsome fraction of a 4-week-old male NAR, a band of about 60 kDa was detected by Western blot analysis in addition to a small amount of normal-sized albumin. The amount of this aberrant albumin increased in male and female NARs during aging and this increase was enhanced by treatment with 3′-MeDAB. No increase of the 56-kDa protein was detected, its detection probably being disturbed by the immunoglobulin used for immunoprecipitation. The other band of 41 kDa expected was also not detected.

DISCUSSION

In this study, we found four species of albumin mRNAs in NARs: normally spliced, −H, −GH, and −HI mRNAs. In 4-week-old NARs, the major species was identified as −H mRNA; normally spliced mRNA constituted about 10% of the total albumin mRNAs at 4 weeks and about 3% at 31 weeks of age (Fig. 1). These results are consistent with the report of Shalaby and Shafritz (6) that −H mRNA is the major species of albumin mRNA in NARs and that normally spliced mRNA constitutes about 1% of the total cytoplasmic albumin mRNAs in rats weighing 100–300 g.

Mutation at the consensus sequence of a 5′ splice site usually causes skipping of an adjacent upstream exon (17–21), activation of cryptic splice sites (17, 22–25), or both (23). The mechanism of this exon skipping was analyzed by Robberson *et al.* (26). According to their model, in NAR, the interaction of exon H with U5 and U2 small nuclear ribonucleoprotein particles (snRNPs) at the 3′ splice site of intron GH may be unstable due to the absence of exon definition with U1 snRNP at the 5′ splice site of intron HI because of 7-base-pair deletion at this site. This may be the mechanism underlying the production of −H mRNA. Exon-skipped mRNAs have been detected at normal levels in several studies (18, 20, 21). Exon H skipping of albumin mRNA results in premature termination of coded protein in exon I. The fact that mRNAs with premature termination codons are unstable (27, 28) may explain why the level of −H mRNA was much lower than that of albumin mRNA in SD rats.

Total mature albumin mRNAs in NARs increased during aging and this increase was enhanced by 3'-MeDAB treatment. Increases of the transcriptional rates or more efficient splicing of transcripts might be involved in their increases. Albumin mRNA in the poly(A)$^+$ fraction is reported not to increase with age for up to 12 months (16), so the observed increase in aged NARs is apparently a characteristic of NARs.

A striking change associated with aging and 3'-MeDAB treatment was the relative increase of mRNAs with two skipped exons among the four species of albumin mRNAs. Two possible mechanisms could be involved in this change. One is an additional mutation in the albumin gene. A newly induced mutation at the 5' site of intron GH and/or the 3' site of intron FG, which causes exon G skipping, or the 3' site of intron HI and/or the 5' site of intron IJ, which causes exon I skipping, could result in production of mRNA with two skipped exons. Actually, a second mutation that resulted in an mRNA with two skipped exons was found (18). Mutations in introns GH and IJ and exons G and H could also be involved (17–21, 23).

The other target for suppressor mutation could be the splicing machinery. Actually, a mutated U1 small nuclear ribonucleoprotein particle induced alternative splicing (29). Possibly some protein factor(s) such as SF2, which influences 5' splice site selection, is involved in two-exon skipping (30). It is interesting that the level of albumin mRNAs larger than normal that hybridized with the probe H increased during aging and their increases were enhanced by a 3'-MeDAB treatment (Fig. 1*C*). Increases of the transcriptional rates or immature mRNA stabilities might be involved in their increases. If the increase of mRNA with two skipped exons is due to alteration of the splicing machinery, NARs should be a good model for analysis of the molecular mechanisms of splicing.

In this study we showed that the amount of mature intact albumin mRNA in 4-week-old NARs was about 1/4000 that in SD rats. It increased 2-fold with age and 3-fold on 3'-MeDAB treatment of aged NARs (Fig. 1*C*). These levels of the mRNA correlated well with those of albumin in serum in young, aged, and 3'-MeDAB-treated aged NARs (2). Thus the normal-sized albumin detected in the cytosolic fraction of NARs is probably secreted into the serum.

An aberrant 60-kDa albumin was detected in the microsomal fraction of aged NARs and its level was increased by 3'-MeDAB treatment of aged NARs. This aberrant albumin may be encoded by −HI mRNA, which was increased in aged and 3'-MeDAB-treated aged NARs. The presence of an aberrant 56-kDa albumin was not proved, due to proximity of a strong band of immunoglobulin, and aberrant 41-kDa albumin, which should be encoded by −H mRNA, was not detected as a specific protein of the NAR. Since 60-kDa protein was detected in only the microsomal fraction, not in the cytosolic fraction or the serum (2), there may be a defect in secretion of this protein into the serum. A recent study by immunoelectron microscopy also showed that in albumin-positive hepatocytes of NARs, albumin accumulates in the rough endoplasmic reticulum and secretory vesicles in addition to Golgi vesicles (31). The increase of the 60-kDa protein could partly explain the increase of the albumin-positive hepatocytes.

Albumin has three domains, each consisting of electrostatically stable helical segments (32, 33). Deletion of amino acids corresponding to exons H and I probably destroys this stable electrostatic state and so the tertiary structure of the aberrant albumin may be changed. Probably this is why 60-kDa albumin was not secreted. This possibility should be examined by studies on the structure of the protein.

Treatment with 3'-MeDAB seems to exaggerate age-associated perturbation of mRNA processing. NARs are useful for studies on the effect of aging on mRNA processing.

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