# Chronic Ethanol Consumption Leads to Destabilization of Rat Liver β-Galactoside α2,6-Sialyltransferase mRNA

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**Chronic ethanol consumption in rats is accompanied by decreased levels of Galβ1,4GlcNAc α2,6-sialyltransferase (2,6-ST) activity in the liver. Our previous studies have shown that there is a concomitant decrease in the levels of 2,6-ST mRNA. In this study, the alteration in the regulation of 2,6-ST expression by chronic ethanol consumption was assessed by Northern hybridization, nuclear run-on experiments, and 2,6-ST mRNA stability studies. 2,6-ST downregulation was found at 4 weeks of feeding an ethanol diet (36% of calories from ethanol) and remained up to 8 weeks. The decrease in 2,6-ST mRNA levels was found to be dose-dependent, with lower dose of ethanol (12% and 24% of total dietary calories from ethanol) being ineffective and the effects being manifested only when 36% of the dietary calories were from ethanol. The effects of chronic ethanol feeding could be completely reversed within 1 week after ethanol consumption was stopped, when 2,6-ST mRNA levels were restored to normal. The downregulation was not sensitive to actinomycin D, indicating that the regulation was not affected at the transcriptional level but at the posttranscriptional level. This was confirmed by nuclear run-on experiments showing that the rate of 2,6-ST mRNA transcription was unaffected by ethanol. Finally, mRNA stability experiments showed that the half-life of 2,6-ST mRNA was reduced 50% in ethanol-fed rat livers compared with control rat livers. Taken together, the results show that 2,6-ST mRNA is regulated at the posttranscriptional level and chronic ethanol intake downregulates 2,6-ST expression by destabilizing its mRNA.**



SIALYLTRANSFERASES (STs) are a family of 10 to 12 glycosyltransferases that catalyze the transfer of sialic acid from its high-energy donor, cytidine monophosphate sialic acid, to the nonreducing terminal positions on the carbohydrate groups of glycoproteins and glycolipids in the Golgi compartment.[1,2] While sialic acids are key determinants of many carbohydrate structures involved in biological recognition events such as host-cell recognition, clearance of asiologlycoproteins from the circulation, cell-cell adhesion during development, and adhesion of leukocytes to endothelial cells, STs generate considerable structural diversity by transferring sialic acid with remarkable specificity for the oligosaccharide substrate.[2] Although STs use a common donor substrate, they exhibit specificity for the sequence of their oligosaccharide acceptor substrate and the anomeric linkage formed between the sialic acid and the sugar to which it is attached.[3] In the liver, four specific STs have been identified as follows: (1) Galβ1, 3 GalNAc α2,3-ST (2,3-ST1), (2) Galβ1,3 GalNAc/Galβ1,4 GlcNAc α2,3-ST (2,3-ST2), (3) Galβ1,3(4) GlcNAc α2,3-ST (2,3-ST3), and (4) Galβ1, 4 GlcNAc α2,6-ST (2,6-ST).[4]

Transferrin and apolipoprotein E are two glycoproteins in the liver with *N*- and *O*-type glycosidic linkage, respectively. We have previously shown that chronic ethanol exposure impairs the glycosylation of both *N*- and *O*-glycosylated proteins in rat liver.[5] We have also shown that the relative sialylation of transferrin and apolipoprotein E was inhibited 86% and 35%, respectively, in long-term alcohol-fed (AN) rats compared with pair-fed control (CN) rats.[5] In this regard, a comparison of the effects of long-term ethanol treatment on key glycosylating enzymes in the liver showed that the activities of mannosyltransferase and galactosyltransferase were decreased 24% and 21%, respectively, whereas the activity of 2,6-ST was decreased markedly by 53% in AN versus CN rats.[5] This inhibitory effect of ethanol was specific for 2,6-ST, since the activities of 2,3-ST3 and 2,3-ST1 were decreased only 32% and 4% (nonsignificant [NS]), respectively, by the same long-term ethanol treatment.[5]

In an effort to understand the molecular events in the liver that lead to the drastic decrease in the activity of 2,6-ST enzyme following chronic ethanol consumption, we have initially shown that this is due to a concomitant decrease in the hepatic synthetic rate of 2,6-ST.[5] Subsequently, we showed that in alcohol-fed rats, there was a similar decrease (59% decrease compared with pair-fed controls) in the 2,6-ST liver mRNA level.[6] The rat 2,6-ST gene is a characteristic gene with tissue-specific alternative splicing leading to different-sized mRNA in different tissues. In this report, we have investigated the mechanism of downregulation of 2,6-ST mRNA by ethanol. We found that chronic ethanol consumption leads to decreased stability of 2,6-ST mRNA, which in turn leads to downregulation.

## MATERIALS AND METHODS

### *Materials*

Actinomycin D was procured from Life Technologies (Gaithersburg, MD). Radioisotopes were purchased from ICN Biomedicals (Alameda, CA). Molecular biology–grade sodium dodecyl sulfate (SDS) was purchased from Bio-Rad (Hercules, CA). All other biochemicals were of the highest grade commercially available.

### *Animals*

Weanling male Wistar Furth rats (Charles River, Wilmington, MA) were maintained on normal rat chow until they reached a body weight of approximately 150 g. Animals were divided into two groups with the required number of rats in each group and pair-fed the respective control (CN) and ethanol (AN) liquid diets for the indicated periods. The diets were isocaloric and were formulated according to the modified method

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of Lieber and DeCarli.[7] Accordingly, 40% of the total energy of the AN diet was from fat, 20% from protein, 36% from ethanol, and 4% from carbohydrate. The corresponding isocaloric CN diet had equicaloric amounts of dextrin maltose in place of ethanol. The following experiments were performed.

### *Effect of Time Course of Ethanol Feeding on 2,6-ST mRNA*

In this experiment, CN (n = 12) and AN (n = 12) rats were fed the respective diets for a total period of 8 weeks. At the end of the first 4 weeks, six rats in each group were killed by aortic exsanguination. The liver was removed and quick-frozen in liquid $N_2$ and stored at −80°C until further analysis. At the end of 8 weeks of feeding, the remaining rats were all killed as just described and the liver was removed and treated exactly as already described.

### *Effect of Ethanol Concentration in the Diet on 2,6-ST mRNA*

This experiment consisted of three groups of six rats each. While one group was fed the control CN diet, the other two groups each were fed with two different concentrations of the ethanol-containing diet. One group received 12% of energy from ethanol, and the other received 24% of energy from ethanol. All three groups were fed for 8 weeks. At the end of 8 weeks, all of the animals were killed by aortic exsanguination and the liver was removed and treated as already described.

### *Effect of Ethanol Withdrawal on 2,6-ST mRNA*

This experiment consisted of five groups of rats with six rats each. One group was fed the control CN diet, and the remaining groups were fed the diet with 36% of energy derived from ethanol. All of the groups were fed for 8 weeks. At the end of 8 weeks, ethanol was gradually withdrawn (to avoid any sudden alcohol withdrawal effects) over a period of 1 week. The rats were then maintained on the control CN diet for 1, 2, 4, and 6 weeks. At the end of each time point, animals were killed by aortic exsanguination and the liver was removed and treated as already described until further analysis.

### *Effect of Ethanol on the Stability of 2,6-ST mRNA in the Rat Liver*

In this experiment, a total of 42 rats were divided into two groups, CN and AN (36% of energy from ethanol), and fed the respective diets for 8 weeks. At the end of 8 weeks, they were all injected with actinomycin D at a dosage of 50 μg/100 g body weight every 12 hours. At the end of 0, 4, 8, 12, 16, 24, and 30 hours of actinomycin D treatment, three rats from each group were killed by aortic exsanguination and the liver was removed and treated as already described until further analysis.

### *Effect of Ethanol on the Rate of Transcription of 2,6-ST mRNA*

*Nuclear run-on assay.* For isolation of nuclei, CN and AN (36% of calories from ethanol) rats (six per group) fed the respective diets for 8 weeks were killed by aortic exsanguination under pentobarbital anesthesia (50 mg/kg intraperitoneally), and 100 mg of the liver was quickly removed and used for isolation of nuclei. The nuclei were prepared as described previously.[8] Nascent $^{32}$P-labeled RNA transcripts were obtained from isolated nuclei by the method of Birch and Schreiber[9] with slight modifications. Nuclei were incubated in a total volume of 350 μL containing 50 mmol/L HEPES, pH 7.5, 50 mmol/L NaCl, 2.5 mmol/L $MgCl_2$, 0.05 mmol/L EDTA, 5 mmol/L dithiothreitol, 1 mmol/L each of adenosine triphosphate, guanosine triphosphate, and cytidine triphosphate, 2 mmol/L creatine phosphate, 2 μg creatine phosphokinase, 20% glycerol, 20 μg heparin, 1 mmol/L spermine, 1 mmol/L EGTA, 0.1 mmol/L phenylmethylsulfone fluoride, 100 U human placental ribonuclease inhibitor, and 125 μCi [$^{32}$P]UTP at 32°C for 30 minutes. After incubation, the reaction mixture was treated with 50 U DNase I for 10 minutes followed by digestion with 100 μg/mL proteinase K and 0.5% SDS for 30 minutes at 37°C. RNA was extracted twice with phenol: chloroform:isoamylalcohol (25:24:1 vol/vol/vol) and precipitated from the aqueous phase by ethanol. Unincorporated [$^{32}$P]UTP was removed by Biogel (Bio-Rad, Hercules, CA) spin-column chromatography. Hybridization of the labeled RNA to linearized plasmid containing 2,6-ST cDNA was performed according to the method of Ausubel et al.[10]

*Isolation of total RNA.* Total RNA from the liver was extracted using Tri-Reagent (Molecular Research Center, Cincinnati, OH). Liver samples of 50 to 100 mg were homogenized in 1 mL Tri-Reagent. The samples were stored for 5 minutes at room temperature followed by addition of 0.2 mL chloroform, shaken vigorously for few seconds, and again stored at room temperature for 15 minutes. This was followed by centrifugation at 12,000 × *g* for 15 to 20 minutes at 4°C. Following centrifugation, the upper aqueous phase was carefully pipetted into a sterile tube, and the RNA was precipitated by addition of 0.5 mL isopropanol and incubated at room temperature for 5 to 10 minutes. The RNA was pelleted by centrifuging again at 12,000 × *g* at 4°C for 15 minutes. The precipitated RNA was washed in 70% ethanol, dried, and quantified spectrophotometrically after solubilization in Formazol, an RNA solubilizing agent (Molecular Research Center).

*Northern blotting.* RNA electrophoresis and Northern transfer onto nylon membranes were performed as described by Ausubel et al.[10] Total RNAs (20 μg) from control rats and ethanol-fed rats were electrophoresed in formaldehyde agarose gels. RNA was transferred to the nylon membrane by overnight transfer in 10× SSC (1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate) as described. Later, the wet nylon membrane was exposed to UV light for fixing the RNA onto the membrane, followed by air-drying. Dried membranes were used for hybridization.

*Dot blotting of RNA.* Where indicated, analysis of total RNA (20 μg) from CN and AN rat livers was performed in 10× SSC per standard procedures.[10]

*Northern hybridization.* cDNA probes were used for Northern hybridization. The probe for 2,6-ST was kindly provided by Dr Joseph Lau, Roswell Park Memorial Cancer Institute, Buffalo, NY. The coding sequence of 2,6-ST was a 780-base pair *Bst*EII–cleaved fragment. A cDNA probe for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was purchased from the American Type Culture Collection (Rockville, MD; ATCC No. 81141). Probes were labeled using a Random Prime Kit (BMB, Indianapolis, IN) and [α-$^{32}$P]dCTP 3,000 Ci/mmol. Hybridizations were performed according to the method of Church and Gilbert.[11] Prehybridization was performed in 10 mL buffer at 62°C for 10 minutes, followed by hybridization with the labeled cDNA probes also at 62°C for 16 to 18 hours in 10 mL hybridization buffer. Probes were added at 1 × $10^6$ cpm/mL hybridization buffer. Later, the blots were washed and used for autoradiography, followed by quantitation of the radioactive spots using a radioanalytic system (AMBIS, San Diego, CA).

## RESULTS

### *2,6-ST Expression in Control and Chronic Alcohol-Treated Rats*

*Effect of time course of ethanol feeding.* Previously, we have shown that 2,6-ST activity is reduced 53% in rat livers treated with alcohol for 8 weeks compared with control rat livers.[5] To examine the molecular basis of this reduction, we used a cDNA probe of the rat liver 2,6-ST mRNA to assess its expression at the mRNA level. To determine if the decreased 2,6-ST activity was due to a corresponding decreased steady-state level of its mRNA, total cellular RNA was isolated from

both control and chronic ethanol-treated (4 weeks and 8 weeks) rat livers. Northern blot analysis showed that 2,6-ST mRNA migrated as a single band consistent with the 4.3 kb mRNA. Exposure of rats to ethanol for either 4 weeks or 8 weeks resulted in a 59% ($P < .001$) decrease in the steady-state level of 2,6-ST mRNA compared with control 2,6-ST mRNA levels (Fig 1). However, there was no change in the level of GAPDH mRNA, a housekeeping gene.

*Effect of concentration of ethanol in the diet.* To assess the dosage-dependent decrease of 2,6-ST mRNA in alcohol-fed rat livers, total RNA was isolated from the liver of rats fed 12%, 24%, and 36% of total calories as ethanol in the diet for 8 weeks and analyzed by Northern blot. The 2,6-ST mRNA signal was quantified using the AMBIS radioanalytic system and is displayed in Fig 2. It is obvious from the figure that the level of 2,6-ST mRNA was affected only when dietary ethanol calories

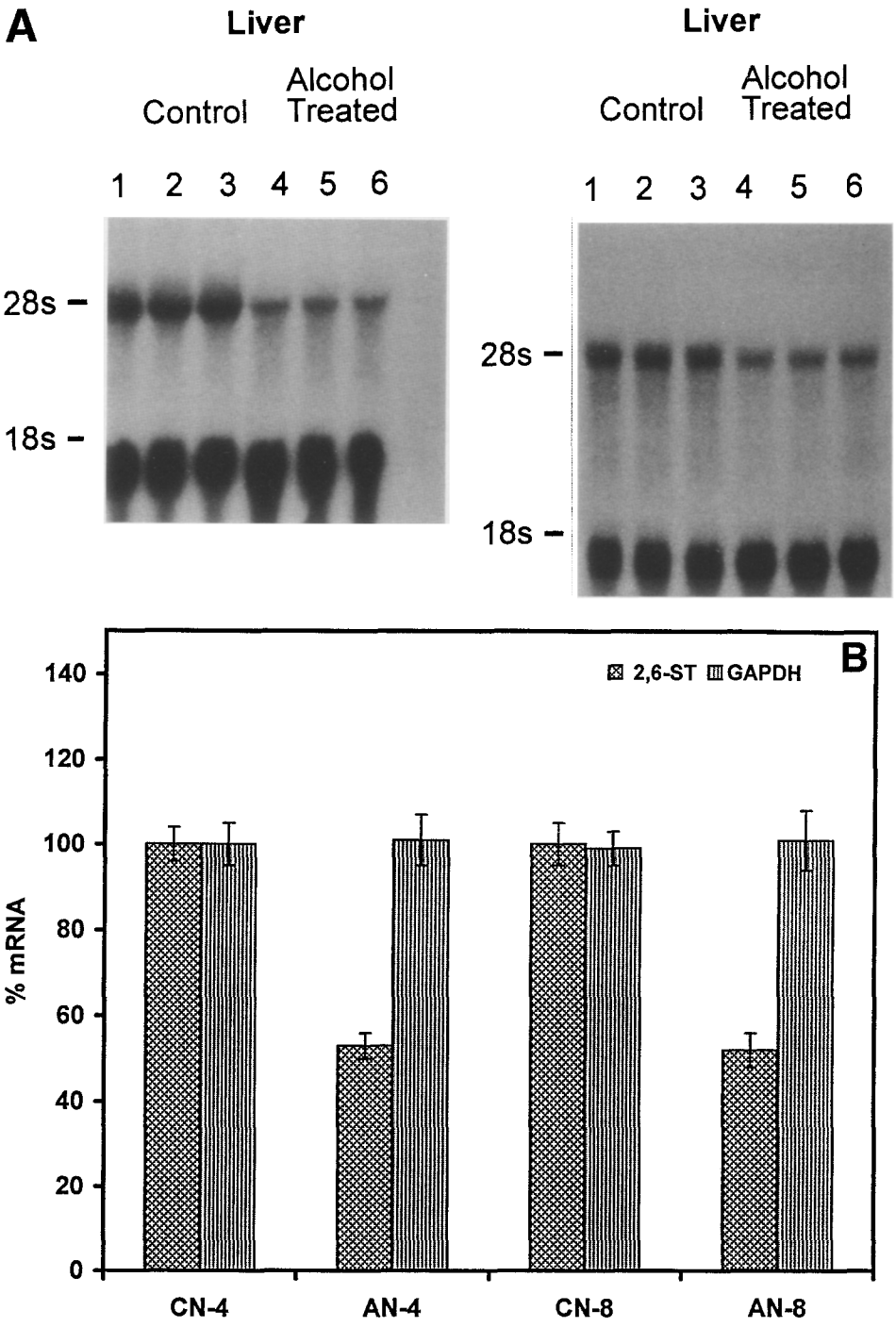


**Fig 1. (A) Effect of chronic ethanol intake (left, 4 weeks; right, 8 weeks) on 2,6-ST mRNA levels in rat liver. Total cellular mRNA was extracted from the liver of 3 control (lanes 1-3) and 3 chronic ethanol-fed (36% of calories from ethanol) rats (lanes 4-6). RNA (20 μg) was electrophoresed on 1% agarose gel in the presence of formaldehyde (2.2 mol/L), transferred to nylon membrane (Magna graph), and hybridized to $^{32}$P-labeled 2,6-ST cDNA probe and GAPDH cDNA probe. Later, the blot was washed and exposed to x-ray film with an intensifying screen for 24 hours, followed by quantitation of the radioactive spots in a radioanalytic system (AMBIS). Data are 1 blot from 3 experiments. (B) Quantified radioactive counts were plotted as % 2,6-ST mRNA considering the counts in control animals as 100%. CN-4 and CN-8, data from rats fed control diet for 4 weeks and 8 weeks, respectively; AN-4 and AN-8, data from rats fed alcohol diet (36% of calories from ethanol) for 4 weeks and 8 weeks, respectively. Each value is the mean ± SE of 3 independent determinations.**

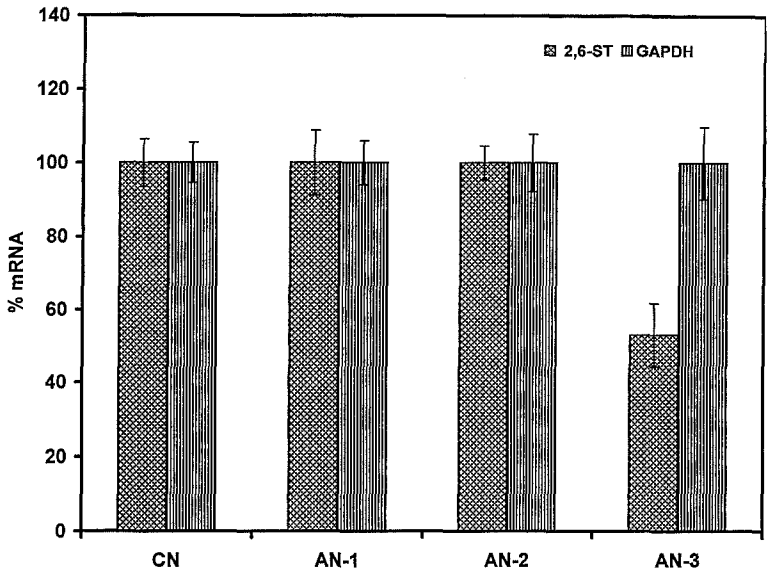


**Fig 2. Dose-response of chronic ethanol intake on ST mRNA in rat liver. Groups of rats were fed either the control diet or the ethanol diet containing ethanol at different dosages, AN-1 (12% of calories from ethanol), AN-2 (24% of calories from ethanol), and AN-3 (36% of calories from ethanol) for 8 weeks. Later, the animals were killed by aortic exsanguination and the liver was removed for preparation of total RNA. RNA (20 μg) from each rat liver was subjected to agarose electrophoresis and hybridized to $^{32}$P-labeled 2,6-ST cDNA probe and GAPDH cDNA probe. Later, the blot was washed and exposed to x-ray film with an intensifying screen for 24 hours, followed by quantitation of the radioactive spots in a radioanalytic system (AMBIS). The quantified radioactive counts were plotted as % mRNA considering the counts in control animals as 100%. Each value is the mean ± SE of 3 independent determinations.**

were at the 36% level. Treatment of rats with a dose of 12% or 24% ethanol calories did not appear to affect the level of 2,6-ST mRNA even after 8 weeks of treatment. Here again, the level of housekeeping gene GAPDH was unaltered.

*Effect of withdrawal of ethanol on 2,6-ST mRNA levels in alcohol-fed rats.* To determine the minimum time required for restoration of 2,6-ST mRNA to normal levels after cessation of alcohol consumption, the level of 2,6-ST mRNA in the liver of

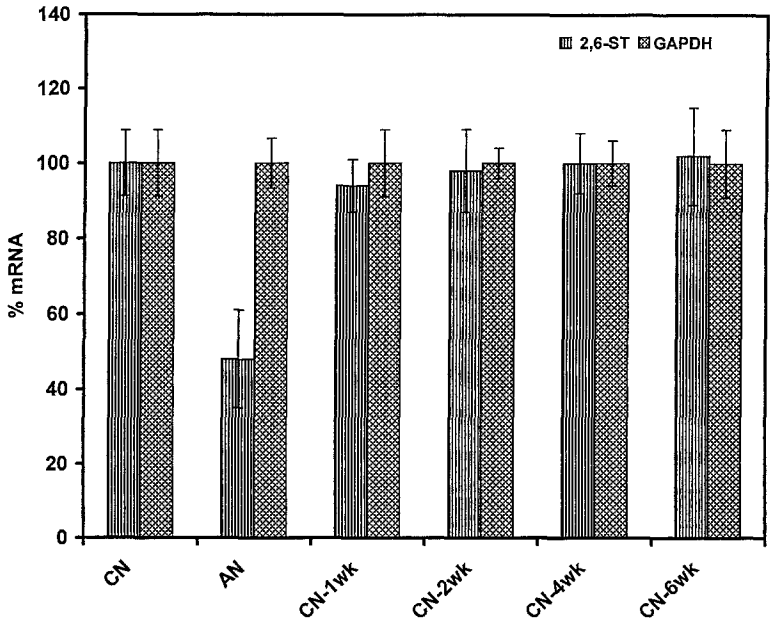


**Fig 3. Effect of withdrawal of ethanol from chronic ethanol-consuming rats on the expression of 2,6-ST mRNA. Groups of rats were fed either the control diet (CN) or chronic ethanol diet (AN) (36% of calories from ethanol) for 8 weeks. This was followed by gradual withdrawal of ethanol from the diet for 1 week. Later, rats withdrawn from the ethanol diet were fed the CN diet for 1, 2, 4, and 6 weeks. At the indicated time points, rats were killed by aortic exsanguination and the liver was removed for preparation of total RNA. RNA (20 μg) from each rat liver was subjected to agarose electrophoresis, hybridization, and quantitation of the radioactive spots as described in Fig 1. The quantified radioactive counts were plotted as % 2,6-ST mRNA considering the counts in control animals as 100%. Each value is the mean ± SE of 3 independent determinations.**

chronic ethanol-fed rats after 1, 2, 4, and 6 weeks of withdrawal of ethanol from the diet was determined by Northern blot analysis of the total RNA (Fig 3). The level of 2,6-ST mRNA was restored to the normal level in about 2 weeks (including 1 week of gradual withdrawal of alcohol) of alcohol withdrawal and continued even after 6 weeks. The level of GAPDH mRNA remained unchanged throughout the experiment in controls and alcohol-fed rats.

*Mechanism of 2,6-ST mRNA Downregulation*

*Rate of transcription of 2,6-ST mRNA.* To determine whether ethanol reduced hepatic 2,6-ST mRNA levels by decreasing the rate of its transcription in the nucleus, a nuclear run-on experiment was performed. Rats were treated with the control or alcohol diet for 8 weeks, followed by removal of the liver. Nuclei were prepared from these livers, and the rate of transcription of 2,6-ST mRNA was determined. The nascent 2,6-ST RNA transcripts in the nuclei were detected by hybridization to dot blots containing denatured stretches of the 2,6-ST cDNA insert (Fig 4). There was no difference in the newly transcribed 2,6-ST mRNA transcripts between control and alcohol-fed rat liver nuclei. The same is true for the housekeeping gene GAPDH. There was no hybridization of the nascent mRNA to a nonspecific sequence of DNA (pGEM-11f plasmid DNA). This indicates that the decrease in 2,6-ST mRNA pools in ethanol-fed rat livers is not due to an inhibition of its rate of mRNA transcription.

*2,6-ST mRNA stability.* To determine whether the reduced level of 2,6-ST mRNA was due to altered mRNA stability, control and alcohol-fed (8 weeks) rats were treated with actinomycin D to inhibit growing chains of ribonucleotides in mRNAs. The rats were killed at various time intervals up to 30 hours after actinomycin D treatment. Later, mRNA levels in all actinomycin D–treated rats were determined by Northern blot analysis, and the 2,6-ST mRNA spots were quantified using the AMBIS radioanalytic system (Fig 5A and B). While 2,6-ST mRNA levels in control livers continued to decrease gradually till 30 hours, the levels in alcohol-fed rats decreased sharply after 12 hours of actinomycin D treatment. Based on the decay curves in control and ethanol-fed rats, the half-life of 2,6-ST mRNA was decreased from 22 hours in the control group to 11 hours in the ethanol-fed group. No such difference in the half-life was found in the case of the housekeeping gene GAPDH mRNA between control and alcohol-fed rats. These results show that, indeed, the decrease in 2,6-ST mRNA levels is a result of a decrease in its stability.

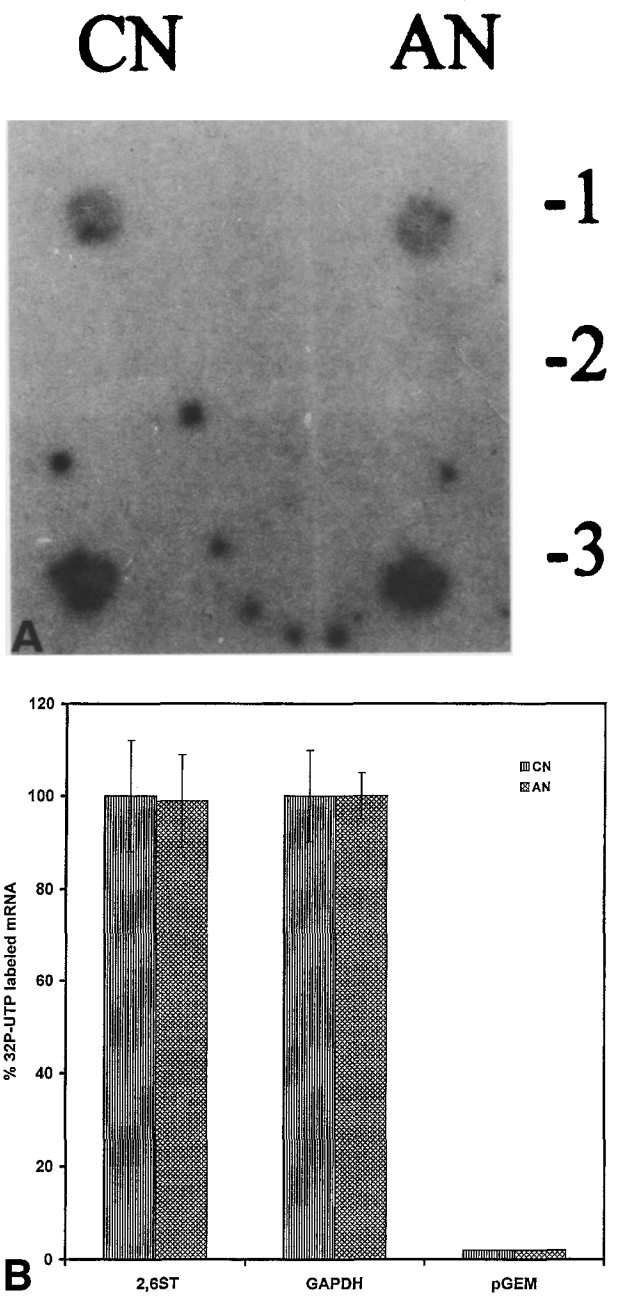


**Fig 4. Effect of chronic ethanol intake on the nuclear transcription rate of 2,6-ST mRNA. (A) Nuclei were isolated from control (CN) or chronic ethanol-fed (36% of calories from ethanol for 8 weeks, AN) rat livers, and nascent $^{32}$P-labeled RNA was prepared. The labeled RNA chains were hybridized to 1 μg 2,6-ST cDNA insert (row 1), 1 μg pGEM11zf vector DNA (row 2), or 1 μg GAPDH cDNA insert (row 3). Data are 1 blot from 2 experiments. (B) Quantified radioactive counts were plotted as % [$^{32}$P]UTP-labeled mRNA considering the counts in the control samples as 100%. Each value is the mean ± SE of 3 independent determinations.**

## DISCUSSION

Ethanol-mediated impaired glycosylation of transferrin leads to the formation of carbohydrate-deficient transferrin (CDT), which is currently used as a viable marker for chronic alcohol consumption.[2] Glycosylation of proteins in the liver is one of the important functions that has many other ramifications, including secretory activities and also immunochemical aspects of liver proteins, since the structures of these glycoproteins and glycolipid sugar chains are determined by the specificity of the glycosyltransferases present. Our ongoing studies have clearly established that ethanol downregulates hepatic 2,6-ST activity and its mRNA.[6]

In this report, we have examined 2,6-ST in further detail to determine the mechanism of this effect at the molecular level. It is unequivocally demonstrated that liver 2,6-ST mRNA levels

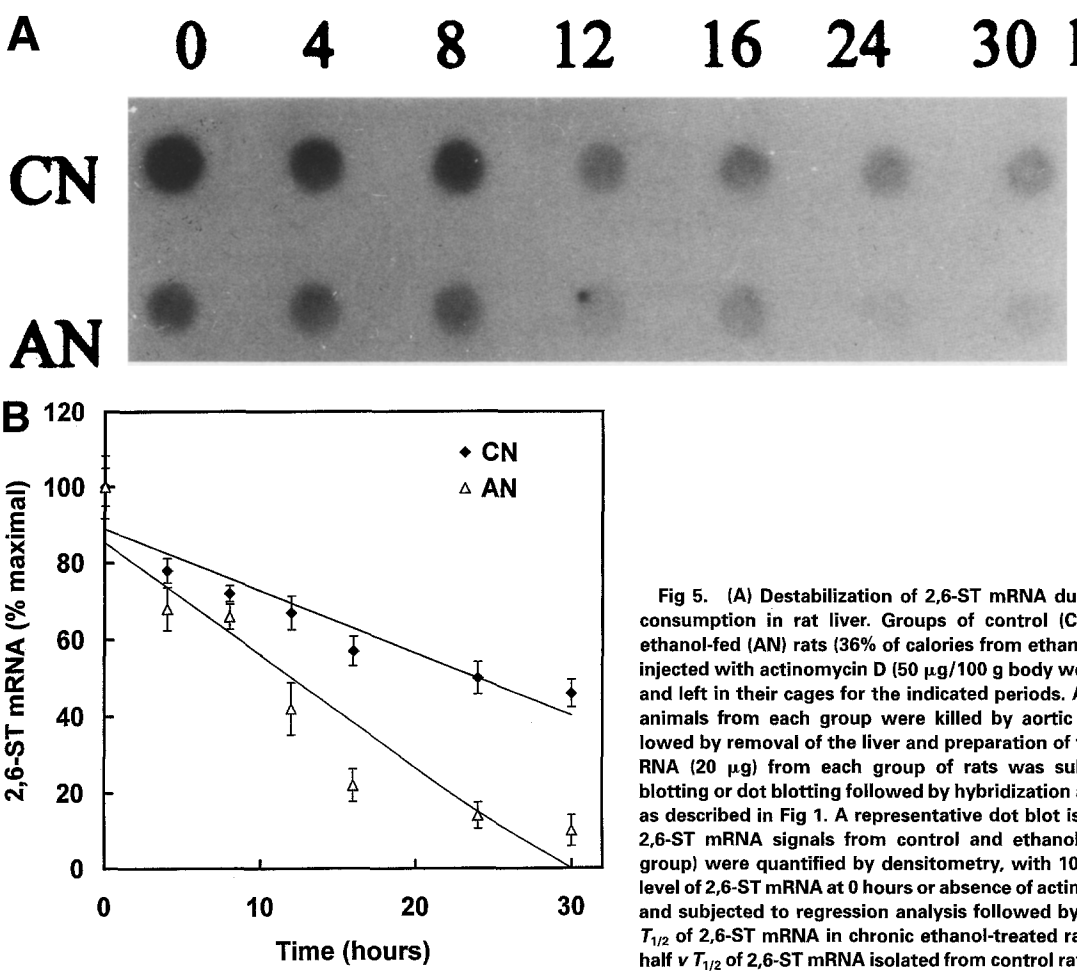


**Fig 5. (A) Destabilization of 2,6-ST mRNA due to chronic ethanol consumption in rat liver. Groups of control (CN) rats and chronic ethanol-fed (AN) rats (36% of calories from ethanol for 8 weeks) were injected with actinomycin D (50 μg/100 g body weight every 12 hours) and left in their cages for the indicated periods. At each time point, 3 animals from each group were killed by aortic exsanguination, followed by removal of the liver and preparation of total liver RNA. Total RNA (20 μg) from each group of rats was subjected to Northern blotting or dot blotting followed by hybridization and autoradiography as described in Fig 1. A representative dot blot is shown. (B) Relative 2,6-ST mRNA signals from control and ethanol groups (3 rats per group) were quantified by densitometry, with 100% representing the level of 2,6-ST mRNA at 0 hours or absence of actinomycin D treatment, and subjected to regression analysis followed by plotting the values. $T_{1/2}$ of 2,6-ST mRNA in chronic ethanol-treated rat liver is reduced by half *v* $T_{1/2}$ of 2,6-ST mRNA isolated from control rat livers.**

decrease by at least 50% even at 4 weeks of ethanol consumption and remain at that level through 8 weeks of ethanol feeding (Fig 1). We further demonstrated that this effect of alcohol is also concentration-dependent, since 2,6-ST mRNA appeared to be at equal levels in control and alcohol-fed rats until the dose of ethanol reached 36% of the total calories of the diet. Lower dosages of ethanol at 12% and 24% of total calories fed for 8 weeks did not seem to have any inhibitory effect on liver 2,6-ST mRNA levels (Fig 2). Significantly, the downregulation of 2,6-ST mRNA is completely reversible by simply removing ethanol from the diet. We found that 2,6-ST mRNA was restored to normal levels as early as 1 to 2 weeks after stopping ethanol feeding (Fig 3). These results imply that alcohol levels less than 36% of dietary calories do not have much effect on 2,6-ST mRNA levels. The observation in the dose-response experiment also lends support to the withdrawal experiment, in that 2,6-ST levels remain unchanged up to a dosage level of alcohol at 24% of total dietary calories. 2,6-ST levels decrease only when alcohol levels are increased up to 36% of total dietary calories.

We speculated that due to the complex nature of the 2,6-ST promoter, ethanol may in fact interfere with the transcription rate. Examination of the rate of transcription of 2,6-ST mRNA in control and ethanol-fed rats showed that ethanol does not appear to interfere with this process. Wang et al[12] have reported that 2,6-ST mRNA can be affected by dexamethasone. There is an upregulation of the gene by dexamethasone reportedly due to an increased rate of transcription. These investigators have reported that there was no difference between control liver cells and dexamethasone-treated liver cells in the transcription start site. On the other hand, Fayos and Bartles[13] have reported that peroxisome proliferators, a structurally diverse group of relatively low–molecular-weight xenobiotic compounds that includes certain hypolipidemic agents and pthalate-ester plasticizers, lead to reduced levels of 2,6-ST and 2,3-ST in rat liver. However, they have not reported the mechanism of the downregulation.

Because of this observation, it was necessary to investigate whether the regulation was at the posttranscriptional level. From the results of mRNA stability experiments, it appears that ethanol (at 36% of energy calories) downregulates 2,6-ST by mRNA destabilization. The half-life of 2,6-ST mRNA was decreased 50% in ethanol-fed rat livers compared with control livers (Fig 5A and B).

The 2,6-ST mRNA and its gene have been well studied by others, and its cDNA has also been cloned. It is interesting that this gene is expressed and spliced in a tissue-specific manner, with a 4.7-kb message expressed in most tissues, an abundant

4.3-kb mRNA in the liver, and a 3.6-kb mRNA in the kidney.[14] It is also interesting that the 2,6-ST mRNA has long stretches of 5′ and 3′ untranslated regions (UTRs). It is clear that regulated changes in mRNA stability play an important role in modulating the level of many eukaryotic genes.[15,16] This appears to be the case for 2,6-ST mRNA, since its half-life is reduced 50% after chronic ethanol feeding. There are reports of several mRNAs, especially those with long stretches of 5′ and 3′ UTRs, being regulated posttranscriptionally, and these mRNAs are known to exist as mRNPs (messenger ribonucleoproteins) with specific proteins bound to the UTRs for stabilization.[17-19] In such cases, 3′-UTR–specific determinants that influence mRNA stability have been identified. These include the adenosine-uridine (AU)-rich domains in the mRNA, for example, the mRNAs of granulocyte/macrophage colony-stimulating factor, c-*myc,* and the characteristic stem-loop structures in histone, ferritin, and transferrin receptor mRNA.[20,21]

Close examination of the 3′-UTR sequence of 2,6-ST mRNA reveals such AU-rich sequences that may have the potential to form stem-loop structures. It is also well known that in such conditions, there are *trans*-acting factors that bind to these stem-loop structures and regulate the stability of an mRNA. Whether such *cis*- or *trans*-acting factors are involved in the stability of 2,6-ST mRNA remains to be seen. This is an entirely different and lengthy investigation that we have just started, and it will be published separately at a later time.

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