Structure and Expression of the Rat c-jun Messenger RNA: Tissue Distribution and Increase during Chemical Hepatocarcinogenesis

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ABSTRACT

c-jun is the cellular homologue of the recently isolated nuclear oncogene v-jun. This protooncogene encodes the cellular transcription factor AP-1. We have isolated the complementary DNA clone of Rat c-jun mRNA. The rat c-jun complementary DNA clone encodes 334 amino acid residues, the sequence of which shows about 98, 96, and 81% homologies with mouse, human, and chicken c-jun products, respectively. The c-jun mRNA is highly expressed in the lung and slightly expressed in the brain. The amount of mRNA is even smaller in the liver and is not detected in the spleen. We have also determined c-jun expression during chemical hepatocarcinogenesis and demonstrated increased expression of mRNA in the precancerous lesion, hyperplastic nodules, as well as in the primary hepatocellular carcinomas.

INTRODUCTION

We have been studying the expression mechanisms of the rat GST-P gene during hepatocarcinogenesis. GST-P is expressed specifically from the early stages of chemical hepatocarcinogenesis to hepatocellular carcinomas in the rat (1, 2). Because of the extremely high coincidence between GST-P expression and (pre)neoplastic transformation of rat hepatocyte, this enzyme has been used widely as an early tumor marker in this system (3–9). In an attempt to study the regulatory elements of the GST-P gene, we have found two copies of TRE-like sequences in the 5′-upstream region of this gene (10, 11). We have also shown that GST-P gene is activated by TPA in a certain cell line (11). Recent reports indicate that transcription factor AP-1, encoded by c-jun gene, binds to TRE sequence and stimulates transcription of the gene containing TRE (12–16). c-jun is transiently activated during G2-M transition, and this expression pattern is quite similar to that of the c-fos and c-myc, which are called the immediate early genes (17, 18). These reports suggest that the c-jun product is closely related to the progression of the cell cycle through G1. More interestingly, another nuclear oncogene c-fos product specifically associates with c-jun product and amplifies the transactivator function against TRE-containing genes (19–21). For further studies of regulation mechanisms of GST-P gene, particularly in terms of the relationship between GST-P gene and the c-jun, we have isolated the cDNA clones of the rat c-jun mRNA. Here, we report the primary structure of rat c-jun mRNA and the deduced amino acid sequence. We also show its tissue distribution and changes in expression during chemical hepatocarcinogenesis.

MATERIALS AND METHODS

cDNA Cloning and Sequencing. A cDNA library against rat liver poly(A)-containing RNA was constructed by using a λgt10 cDNA cloning kit (Amersham, United Kingdom) according to the protocol recommended by the supplier. The cDNA library was screened with synthetic oligodeoxynucleotide probes complementary to the human c-jun cDNA. Two of the 36-mer oligodeoxynucleotides complementary to the nucleotide positions from 469 to 504 (NH2-terminal probe) and from 1286 to 1321 (COOH-terminal probe) of the human c-jun cDNA clone ch-J1 and ch-J2 isolated by Angel et al. (16) were synthesized and used for the screening. The synthetic probes were hyridized to a phage cDNA library immobilized on the filters in 1 M NaCl-20 mm Tris-HCl pH 8.0-1 mm EDTA-0.1% SDS, 10× Denhardt’s solution at 50°C and washed in 2× SSC-0.1% SDS at 50°C. The isolated cDNA clones pRJ51 and pRJ101 were sequenced by the dyeoxy method (22).

Chemical Hepatocarcinogenesis. The model of Solt and Farber (23) was used for the induction of hyperplastic nodules in rat liver. Briefly, 200 mg/kg body weight of DEN was injected i.p. into rats and the 0.02% N-2-acetylaminofluorene in standard diet was given for 2 weeks thereafter. Partial hepatectomy was done 3 weeks after DEN injection. Hepatocellular carcinomas were induced either by a diet containing 0.03% AAF or by 0.01% DEN in drinking water (1).

Southern Blot Analysis. Restriction endonuclease-digested rat liver RNA was screened as described in “Materials and Methods.”

RESULTS

cDNA Cloning and Primary Structure of Rat c-jun mRNA. A cDNA library constructed from rat liver poly(A)-containing RNA was screened as described in “Materials and Methods.” Six plaques of 2×104 plaques were positively hybridized with both COOH-terminal and NH2-terminal probes. Two of the clones contained a 2.0-kilobase insert and four had a 2.6-kilobase insert. One of the clones containing a 2-kilobase insert (pRJ101) and another containing a 2.6-kilobase insert (pRJ51) were chosen for sequencing. In the pRJ101 clone, 553 nucleotides of 3′-terminal portion were missing as compared to the pRJ51. The nucleotide and deduced amino acid sequences are shown in Fig. 1A and the restriction maps in Fig. 1B. The pRJ51 clone containing 2579 nucleotides has a 1002-nucleotide open reading frame that encodes 334 amino acids. The calculated molecular weight of the protein encoded by pRJ51 is 36,000.35. The deduced amino acid sequence of pRJ51 is highly homologous to those of human, mouse, and chicken c-jun products. Comparison of the c-jun amino acid sequences of
the amino acids of the COOH terminus of the c-jun products are (119th-334th) are almost completely identical among these species and viral Jun. These results suggest that about 100 amino acids, which is the major difference between v- and c-jun sequences, correspond to the major determinant of the oncogenicity of the Jun gene, as discussed by Nishimura and associates (17, 18). The NH2-terminal 60-amino acid sequences are highly conserved among these four species, i.e., homologies with humans, mouse, and chicken are 88, 96, and 55%, respectively. However, these regions are relatively diverged, i.e., homologies of the rat c-jun product with those of chicken are 96, 98 and 81%, respectively. 

These results suggest that the tumor suppressor role of Jun might be related to the amino acid sequence at the COOH terminus. In the 3' noncoding region, there are two poly(A) addition signals located at 2005 and 2554. The short mRNA which used the upstream poly(A) signal corresponds to the pRJ1 clone. The sequence related to instability of mRNA, ATTTA (27), is located at 2059 and 2533. No such sequence is found in shorter mRNAs (corresponding to pRJ1 but there are some related sequences such as GTTTA and T clusters.

**Southern Blot Analysis of the c-jun Gene.** Southern blot analysis of the rat genomic DNA using the rat c-jun cDNA as a probe detected a single band in EcoRI, BamHI, and HindIII digests (Fig. 3). The EcoRI digest shows a band of about 2.5 kilobases which is very close to the size of the cDNA clone pRJ51 and mRNA (Fig. 4). Since no EcoRI site is present in the cDNA clone (except for attached linker sequences on both ends), it is unlikely that the c-jun gene has any long intron sequence. Indeed, no EcoRI sites are reported in human, mouse, or chicken c-jun genes (25, 28). A single band was detected under highly stringent washing conditions, suggesting that the c-jun gene is unique in the rat genome. However, many extra bands were detected with lower stringency washing conditions. This suggests that there are many c-jun-related genes or sequences present in the rat genome. Some cDNA clones related to c-jun mRNA were also isolated (29).

**Expression of c-jun in Rat Tissues.** To determine the mRNA levels of c-jun in rat tissues, total RNAs were extracted from the liver, brain, spleen, testis, and lung and analyzed by Northern blot hybridization using pRJ51 as a probe. As shown in Fig. 4A, this gene is most actively expressed in the lung. A
small amount of mRNA was also detected in the brain. A very small amount of mRNA was detected by an extended exposure in the liver and testis but not in spleen. The amounts of c-jun mRNA in tissue culture cell lines and ascites hepatoma cell lines are shown in Fig. 4B. All rat hepatoma cell lines examined including AH130, Morris hepatoma 5123D, and dRLh84 highly expressed c-jun mRNA. HeLa cells contained relatively small amounts of c-jun mRNA. Undifferentiated F9 embryonal carcinoma cells did not have detectable amounts of c-jun mRNA. It has been reported that there was no AP-1 protein (c-jun product) in the undifferentiated F9 cell extract as determined by gel mobility shift assay and by foot printing analysis (30). The nucleotide sequence of rat c-jun cDNA is about 90% homologous with that of the human c-jun cDNA (amino acid coding region) and there are 90-nucleotide and 60-nucleotide stretches that are completely identical with each other. Therefore, it is inferred that the human c-jun mRNA was detected almost at the same efficiency as rat mRNA under the conditions used in these experiments. It should be noted that the experiments shown in Fig. 4 were done separately with different specific activity probes, different amounts of RNA applied, and different times of exposure to X-ray film, so that they are not directly comparable. From the results of other experiments, the hepatoma cell line dRLh84 cells appear to contain 2 or 3 times more c-jun mRNA than lung tissue.

Expression of c-jun mRNA during Chemical Hepatocarcinogenesis. Since all the hepatoma cell lines thus far studied highly expressed the c-jun mRNA, we now determined the expression of c-jun mRNA in early stages of the chemical hepatocarcinogenesis. According to the protocol of Solt and Farber, preneoplastic lesion hyperplastic nodules appeared 4-5 weeks after DEN administration. The RNAs from 4 and 6 weeks after DEN treatment were isolated as described in "Materials and Methods." The RNAs from primary hepatocarcinomas induced by N2-acetylaminofluorene or DEN were also isolated. Fig. 5 shows Northern blot analysis of these RNAs hybridized with a jun probe. For controls of RNA quantitation and transfer, we have rehybridized the same filter with β-actin probe. The intensities of β-actin mRNA bands were almost similar in normal liver and hyperplastic nodule-bearing liver. Some of the primary hepatomas and the hepatoma cell line dRLh84, however, show significantly strong bands, although almost the same amounts of RNAs were applied. This may be because the β-actin gene contains serum-responsive element and rapidly growing hepatoma cells express higher amounts of β-actin mRNA than normal cells (31). At the 4th or 6th week after chemical carcinogen administration, c-jun mRNA in the treated liver was increased substantially as compared with normal liver. All of the five independently induced primary hepatocarcinomas expressed relatively high amounts of c-jun mRNA. In the hyperplastic nodules-bearing liver, however, nodule foci occupied approximately 15 and 60% of the whole liver mass at 4 and 6 weeks after carcinogen treatment (32), respectively.
TRE-like sequence of GST-P gene. These findings, together
analysis suggested that an AP-1-like factor bound with the
amounts of both mRNAs. The GST-P gene has two TRE-like
well that of the GST-P mRNA (36); e.g., the lung expresses the
of lung cells are really active in c-jun expression remain to be
discussed in previous reports (34, 35).

DISCUSSION

Cloning of the Rat c-jun cDNA. We have cloned and se-
quenced rat c-jun cDNA. The transactivator function of cloned
cDNA was confirmed by cotransfection experiments as follows.
The cDNA insert of pRJ101 was joined to an expression vector
containing human /3-actin gene promoter and enhancer complex
(33). This expression plasmid DNA and an indicator plasmid
containing bacterial chloramphenicol acetyltransferase gene
hooked to the human collagenase gene with a TRE sequence
were cotransfected into the undifferentiated F9 cells. The chlor-
amphenicol acetyltransferase activities of the transfected cells
were demonstrated only after introduction of our c-jun expres-
sion plasmid DNA (data not shown).

Comparison of the amino acid sequences between rat, mouse,
human, and chicken indicates a very high conservation of this
protein. Especially 100 amino acids of COOH-terminal domain
human, and chicken indicates a very high conservation of this

Distribution of c-jun mRNA. Among the rat tissues studied,
the lung most actively expressed the c-jun although what types
of lung cells are really active in c-jun expression remain to be
seen. The tissue distribution of the c-jun mRNA parallels fairly
well that of the GST-P mRNA (36); e.g., the lung expresses the
highest amount of both c-jun and GST-P mRNA whereas
normal tissue and precancerous liver, primary hepatocellular
carcinoma, and some hepatoma cell lines express very high
amounts of both mRNAs. The GST-P gene has two TRE-like
sequences in its 5'-flanking region and is stimulated by TPA in
a certain cell line (11). Gel mobility shift assay and footprint
analysis suggested that an AP-1-like factor bound with the
TRE-like sequence of GST-P gene.* These findings, together

with the distribution of the GST-P mRNA and c-jun mRNA,
suggest that the GST-P gene is regulated in major part by
transcription factor AP-1 (c-jun product). On the other hand,
the amount of c-jun mRNA or AP-1 protein alone cannot
always explain the GST-P gene expression. For instance, an
ascites hepatoma, AH130, expresses c-jun mRNA as much as
a Morris hepatoma 5123D does. However, the former expresses
almost no GST-P mRNA (36), while the latter expresses ex-
traordinary amounts of it. Previously, we found that the GST-
P gene having TRE-like sequence in its enhancer was highly
expressed in the hyperplastic nodules as well as in the hepato-
cellular carcinomas. We now found that c-jun was also elevated
in the early stages of liver carcinomas. To what extent, however,
the c-jun expression during hepatocarcinogenesis is involved in
the dramatic activation of GST-P gene remains to be deter-
mined.

In any event, the c-jun cDNA clone described here will
facilitate the elucidation of the mechanism by which specific
expression of the genes occurs that are related to neoplastic
transformation and cell growth.

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