Modulation of Hepatic mRNA Translation Activity and Specific Expression of Arylsulffotransferase IV during Acetylaminofluorene-induced Rat Hepatocarcinogenesis

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ABSTRACT

Enzymatic sulfation of \( N \)-hydroxylated arylamines by mammalian hepatic cytosolic sulfo transferases (AST; EC 2.8.2.1) is an important metabolic step which generates ultimate carcinogens. The metabolic activity of AST IV, the putative isozyme form of AST primarily responsible for catalyzing \( N \)-hydroxy-2-acetylaminofluorene sulfation, is modulated during 2-acetylaminofluorene (AAF)-induced rat hepatocarcinogenesis.

To characterize the molecular mechanisms regulating the differential expression of AST IV, we have assessed polyadenylated mRNA derived from the livers of Sprague-Dawley rats undergoing different stages of AAF hepatocarcinogenesis for general in vitro translation capacity and specific expression of AST IV and albumin. Following 1 and 3 cycles of a cyclical feeding regimen (3 weeks 0.05% AAF, then 1 week basal diet), the mRNA capacity for translation was lowered and the expression of AST IV and albumin was down-regulated about 2-fold each but recovered to normal levels when treated rats were subsequently placed on basal diet for 3 continuous weeks. Cytosolic albumin levels were determined by Western blot analysis to be lowered about 1.5-2-fold.

In contrast, however, mRNA from rats on basal diets for 3 weeks subsequent to cycle 5 of the feeding regimen recovered only about 50% of the capacity for AST IV expression, although overall translation capacity and albumin expression returned to normal levels. This pattern of reversible expression, followed by irreversible expression of AST IV at early and late stages of AAF hepatocarcinogenesis, respectively, provides the first evidence correlating the modulation of hepatic mRNA capacity for AST IV expression with differential cytosolic AST IV activity in the AAF hepatocarcinogenesis model. The results further suggest that during early stages in hepatocarcinogenesis modulation of mRNA protein synthesis functions may be a critical factor in AAF-mediated lowering of AST IV expression, while other persistent genetic lesions are likely playing a more significant role at the late stages of the carcinogenic process leading to neoplastic transformation of initiated hepatocytes.

INTRODUCTION

Numerous chemical carcinogens, such as arylamines, require metabolic activation catalyzed by the enzymatic apparatus of mammalian hepatic cells to exert their genotoxic effects (1–3). Recently, there has been renewed interest in carcinogen-metabolizing enzymes with regard to correlations between the level of enzyme expression and individual differences in susceptibility to the genotoxic effects of chemical carcinogens. The metabolic and toxicity of the hepatocarcinogen AAF in mammals, for example, have been studied extensively and shown to be metabolized to NOHAAF by the microsomal cytochrome P-450 enzyme system (4, 5). The \( N \)-hydroxyl derivative is a proximate carcinogen and also a substrate for a number of other metabolic activating enzymes including deacetylase (6, 7), \( N \)-O-acetyltransferase (7, 8), OAT (9, 10), and arylsulfotransferase (11) which catalyzes \( 3' \)-phosphoadenosine 5'-'phosphosulfate-dependent sulfation reactions. The sulfation of NOHAAF generates an ultimate carcinogen, a highly reactive sulfuric acid ester, capable of reacting with proteins, while forming primarily AAF adducts with RNA and DNA (12–14) to initiate the carcinogenic process (1). Variations in the hepatic levels of \( 3' \)-phosphoadenosine 5'-'phosphosulfate-dependent NOHAAF AST in the Sprague-Dawley rat strain are uniquely correlated with susceptibility to the induction of liver cancer, as well as the critical transition events which facilitate AAF-induced hepatocarcinogenesis (13, 15–17). Relatively higher hepatic AST activity in male rats appears to predispose them for AAF-induced hepatocarcinogenesis (15) while their female counterparts with little or no activity (18) are resistant.

Similarly, there have been extensive studies on the role of the acetylation reactions and the consequence of polymorphic enzyme expression on individual differences in susceptibility to arylamine-induced cancers. Humans and several other mammalian species exhibit a well-defined genetic variation in the capacity for acetylation of carcinogenic arylamines (19, 20). The molecular rearrangement of arylhydroxamic acids by OAT (9, 10) is thought to be a metabolic activation step in the generation of DNA-binding ultimate carcinogens in mammalian colon (9, 21, 22). The levels of OAT activity are reported to parallel high levels of \( N \)-acetyltransferase activity in human and hamster tissues (23–26) and the colonic mucosa activities are thought to be catalyzed by the same acetyltransferase isozyme (23, 27). Thus, based on biochemical studies and epidemiological observations, it has been suggested that genetically determined rapid acetylator status may predispose individuals to arylamine-induced colon cancer (23, 28).

In contrast to the wide knowledge of the biochemical mechanisms involved in the metabolic biotransformation of xenobiotics and especially the carcinogenic activation of AAF, little is still known about the specific molecular mechanisms by which AAF alters the expression of metabolic enzymes and the relevance to the process of carcinogenesis. Furthermore, although most studies on the biochemical and morphological alterations in chemically induced carcinogenesis often look at the early and late stages of the process (29), the present study has also examined intermediate stages considered important for the critical transition events in AAF-induced hepatocarcinogenesis.

In a recent study by Ringer et al. (30), persistent and nonpersistent losses in rat liver AST IV expression were reported to occur during AAF-induced hepatocarcinogenesis, when rats passed from an early stage of low risk to a late stage of high risk for developing liver cancer, respectively. The molecular mechanisms remain to be determined for the loss in AST IV.
expression during the AAF treatment and the reason for persistent loss at the late stage of hepatocarcinogenesis. As an initial step toward making this assessment, it is necessary to determine whether AST IV expression is depressed due to direct effects on protein synthetic machinery, increased protein turnover, or effects on nuclear transcription. The expression of albumin by hepatic mRNA was also assessed because it is known to be constitutively expressed in rat liver (31). Evidence is presented that AAF modulates hepatic mRNA translation activity, which may account for lowered AST IV expression during the early stages of hepatocarcinogenesis, but this effect is, very likely, coupled to other genetic lesions at the late stages.

MATERIALS AND METHODS

Animals. Male and female Sprague-Dawley rats (~200 g) were purchased from Sasco Inc., Omaha, NE.

AAF Feeding Regimen. Male rats were given an AAF feeding regimen modified from the model of Teetor and Becker (32) for inducing hepatocarcinogenesis. This involves cyclical administration of a diet composed of a basal semisynthetic control diet supplemented with 0.05% AAF (17) for 3 weeks followed by basal diet for 1 week. This cyclical feeding regimen is repeated up to 5 cycles while age-matched control rats were maintained on continuous basal diet.

Isolation and Biochemical Characterization of AST IV. The procedures used for isolating, purifying, and characterizing AST IV with respect to purity and enzymatic activity are described in detail in previous studies reported by Ringer et al. (30). A modification of the final step in purification involved substitution of fast performance liquid chromatography (Pharmacia/LKB Biotechnology, Piscataway, NJ) for the SDS-PAGE step. The chromatography was performed with a Mono Q column, with a gradient of 0.0 to 0.2 M NaCl in a buffer of 25 mM Tris, pH 8.0. This step generated native AST IV protein for use in antibody production.

Production of Antibody against AST IV. Purified native AST IV was used to elicit antibody in rabbits as described by Sigel et al. (33). Preimmunization antisera was collected from rabbit and used in negative control assays. Antiserum to the purified AST IV was characterized as described by Ringer et al. (30) and shown to be monospecific for the detection of AST IV in rat liver cytosols. Pure IgG fraction was isolated from the antiserum by affinity chromatography on a protein G-Sepharose column (Pharmacia/LKB Biotechnology, Piscataway, NJ), binding approximately 20 mg IgG/ml of gel matrix.

Extraction of RNA from Rat Liver and Isolation of Poly(A)* mRNA. Total cellular RNA was isolated from the liver of rats at different intervals during the AAF feeding regimen by the guanidinium thiocyanate extraction method developed by Chirgwin et al. (34). The RNA isolated was treated in diethyl pyrocarbonate-treated water, stored frozen at ~70°C. Advantage taken of the characteristic feature of posttranscriptional polyadenylation in the biogenesis of most eukaryotic mRNAs to enrich for poly(A)* mRNA, by the oligo(dT)-cellulose method of Aviv and Leder (35). The amount of total cellular RNA extracted as well as mRNA isolated was estimated by spectrophotometric analysis, using a conversion factor of 25 A260 units/ml RNA. Typical extractions yielded mRNA constituting about 2-5% of total RNA applied to column.

In Vitro Translation of Poly(A)* mRNA and Estimation of Translation Capacity. The method described by Pelham and Jackson (36), which utilizes a nuclease-treated cell-free rabbit reticulocyte system, was used in this study for in vitro translation. The system translates exogenous mRNA with an efficiency approaching that of untreated lysates in vivo (36) and can, therefore, be reliably used for assessing carcinogen-mediated changes in the expression pattern of specific proteins.

In the presence of rat hepatic mRNA the incorporation of [35S]-methionine (Du Pont Research Products, Westwood, MA) into translation products was used to assess the capacity for protein synthesis. The overall capacity of mRNA samples for in vitro translation was estimated from a 1-μl aliquot of products spotted on a 33-mm glass fiber Whatman filter paper (Whatman Int. Ltd., Maidstone, England). The filter paper was treated with trichloroacetic acid, followed by two rounds of sequential washings with each of water, ethanol, and acetone. The filter paper was then air dried and the radioactivity generated by [35S]methionine incorporation into polypeptides trapped on the filter paper was estimated by liquid scintillation counting. Values used as the index of translation activity were derived as described under "Analysis of Data."

Characterization and Evaluation of In Vitro Translation Products for Albumin and AST IV Synthesis. The presence of albumin or AST IV in the in vitro translation products generated by rat hepatic mRNAs was determined by subjecting the products to an immunoprecipitation procedure described in the assay kit supplied by Genex Corporation (Gaithersburg, MD). Initially, products generated by the translation of equal amounts of mRNA were adjusted to equivalent radioactivity contents before immunoprecipitation. Briefly, the immunoprecipitation procedure involved mixing in a microfuge tube 5 μg of primary antibody (anti-albumin or anti-AST IV IgG), 50 μl of a protein G-agarose complex, and phosphate-buffered saline to give a final volume of 200 μl. The mixture was incubated at 4°C for 30 min with gentle end-to-end agitation. Following incubation, radiolabeled in vitro translation product (in volumes not exceeding 50 μl) was added and incubation at 4°C was resumed for another 4 h with gentle agitation. The immunoprecipitate complex formed during the incubation period was pelleted by centrifugation at 10,000 × g for 10 min. The precipitate was subjected to five rounds of stringent washes in 500 μl of phosphate-buffered saline-azide supplemented with 1% Triton X-100 and 0.04% SDS. The final pellet was resuspended in 40 μl of sample buffer made up of 0.1 M Tris (pH 6.8), 2% SDS, and 40% glycerol; heated at 90°C for 10 min; and then centrifuged at 10,000 × g for 2 min to separate protein G-agarose from the immune complex. The supernatant was carefully aspirated and electrophoresed on a 10% SDS-polyacrylamide gel. The relative mobility of specific immunoprecipitated protein bands and molecular weight standards (Du Pont Research Products, Westwood, MA) were assessed both by autoradiography and scanning of dried gels with a radioanalytical imaging system (Ambis Co., San Diego, CA). Translation products generated by mRNA of control rats were immunoprecipitated with nonimmune serum, simultaneously electrophoresed, and used as negative controls. Values for protein levels detected were computed as a ratio of the radioactivity detected in specific gel bands for AST IV or albumin to that in an equivalent region on the negative control lane.

Estimation of Levels of Albumin in Liver Cytosol of Rats at Different Intervals during AAF Hepatocarcinogenesis. Liver tissues were excised from rats at time intervals equivalent to those used in the assessment of mRNA in vitro translation activity during the AAF hepatocarcinogenesis feeding regimen. Hepatic cytosol fractions were isolated as described previously and 4 mg were fractionated on a 10-15% SDS-polyacrylamide gradient gel (Phast System, Pharmacia/LKB Biotechnology, Piscataway, NJ). Separated proteins were transferred to Immobilon-P membrane (Millipore Corp., Bedford, MA) with the aid of a Trans-Blot apparatus (Model 250; Bio-Rad, Richmond, CA). This was followed by immunospecific detection of albumin using a primary IgG antibody against albumin (Cappell Co., Westchester, PA) and a peroxidase-conjugated anti-rabbit IgG (Bio-Rad) secondary antibody. Positive signals, localized as purple bands on the filter paper, were generated by a peroxidase-catalyzed reaction between hydrogen peroxide and 4-chloro-1-naphthol. The filter paper was scanned at 633 nm (LKB Laser Densitometer) and band intensities integrated as absorbance units/μm were used as index of albumin levels in liver cytosols.

Analysis of Data.

Radioactivity incorporated in total translation products
Translation capacity = Radioactivity endogenously incorporated in negative controls
Capacity for AST IV expression = cpm in AST IV immunoprecipitate band
                   -ogui
                     cpm in equivalent band
                   region of control lane

505
RESULTS

Effect of AAF Treatment on Overall Translation Activity of Hepatic Poly(A)* mRNA. Previous studies by Ringer et al. (30) have shown that 0.05% AAF administered to male Sprague-Dawley rats in a cyclical feeding regimen mediates about 80–85% lowering of hepatic cytosol NOHAAF AST IV activity, which is fully recovered when rats are returned to basal diet. However, after the fifth cycle of the feeding regimen, irreversible lowering of the enzyme activity was observed during the recovery period. Subsequent immunochemical analysis of cytosolic AST IV expression showed a pattern which closely paralleled that of the biochemical activity at different intervals in the feeding regimen. In the present study, Fig. 1 shows the translation capacity of equivalent amounts of hepatic poly(A)* mRNA derived from rats at different intervals during rat AAF hepatocarcinogenesis. AAF mediated a lowering of the translation activity of mRNA from rats just completing 3-week AAF diet (weeks 3, 11, and 19), as shown in Fig. 1. When treated rats were administered basal diet for 3 weeks following cycles 1, 3, and 5 (weeks 6, 14, and 12) of the feeding regimen, rapid and full recovery of translation activity was observed (Fig. 1). The nodule fraction also exhibited full recovery of translation activity (Fig. 1). It was also observed that the translation activity of mRNA derived from rats on continuous basal diet at 22 weeks was about 17% lower than for young rats (week 0). Thus, when the translation capacity of mRNA from rats at cycle 5 of the feeding regimen was adjusted relative to age-matched controls, values obtained were about 85% of normal translation activity, while mRNA of treated rats subsequently placed on basal diet for 3 weeks (week 22) showed full (100%) recovery (Fig. 1).

In Vitro Expression of AST IV by Hepatic Poly(A)* mRNA Derived from Rats Given AAF. The AST IV contents of translation products generated by hepatic poly(A)* mRNA isolated from rats at different stages of hepatocarcinogenesis were evaluated following immunoprecipitation and subsequent SDS-PAGE (Fig. 2). At the early stages of the rat AAF hepatocarcinogenesis, administration of AAF for 3 weeks (cycle 1) mediated a 50% loss in the amount of AST IV generated in vitro translation of hepatic mRNA (Fig. 2). When cycle 1- and 3-treated rats were returned to basal diet for 3 weeks (weeks 6 and 14), AST IV expression was recovered to about 77 and 80% of normal levels, respectively. However, in the late stage of rat AAF hepatocarcinogenesis as represented by cycle 5, only 50% of the normal level of AST IV expression was recovered by mRNA of rats subsequently fed basal diet for 3 weeks (Figs. 2 and 3; Table 1). The age-adjusted levels of AST IV expressed by mRNA from post-cycle 5-treated rats (weeks 22 or 25) were 58% for total liver tissue and 39% for isolated large hyperplastic nodules (Fig. 2; Table 1).

In order to further confirm the specificity of the antibody IgG for AST IV, homogenously purified AST IV protein, immunoprecipitated AST IV, and immunoprecipitate from in vitro translation products generated by mRNA of basal rat were concurrently subjected to SDS-PAGE analysis. The immunoprecipitated AST IV comigrated with the pure protein fraction (data not shown) to the M, 32,000–35,000 range, similar to the range reported for NOHAAF AST and AST IV by other investigators (18, 30, 37).

Effect of AAF Administration to Rats on Albumin in Vitro Cytosolic Levels and Poly(A)* mRNA in Vitro Translation Levels. In adult mammals, albumin is known to be produced at high and constant levels (31). However, it has been reported that the levels of synthesis and secretion of albumin are frequently altered in chemically induced primary hepatomas and in transplantable hepatoma cell lines (38–40). We were, therefore, interested in determining whether a pattern of altered albumin expression, paralleling that for AST IV expression, existed in the hepatocarcinogenesis model used in the present study. The data in Fig. 3 show that the capacity of mRNA from rats that had undergone AAF administration for 3 weeks was depressed to about 62% of normal levels. This depression was followed by rapid recovery of over 100% capacity for albumin expression when treated rats are fed basal diet for an additional 3 weeks after treatment (week 6). Similar patterns of loss and recovery were observed at cycles 3 and 5 of the feeding regimen (weeks 11 and 22). Interestingly, the level of albumin expressed by in vitro translation of nodule mRNA was only about 80% of age-matched control rats (Fig. 3; Table 1). Determinations of albumin levels in rat liver cytosol at different intervals in the hepatocarcinogenesis model showed a pattern of depression.

Table 1 Differences associated with age, gender, and AAF treatment in the capacity for in vitro translation and specific expression of AST IV and albumin by rat hepatic mRNA

<table>
<thead>
<tr>
<th>Source of mRNA</th>
<th>Translation capacity*</th>
<th>Levels of AST IV expressed in vitro*</th>
<th>Levels of albumin expressed in vitro*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young male rats</td>
<td>7.42 ± 1.23</td>
<td>4.8</td>
<td>10.4</td>
</tr>
<tr>
<td>Young female rats</td>
<td>6.10 ± 0.10</td>
<td>ND*</td>
<td>8.2</td>
</tr>
<tr>
<td>Old male rats</td>
<td>6.79 ± 0.14</td>
<td>4.2</td>
<td>7.9</td>
</tr>
<tr>
<td>Nodules†</td>
<td>6.27 ± 1.05</td>
<td>2.4</td>
<td>6.2</td>
</tr>
</tbody>
</table>

*Mean ± SD.

†No detectable level of AST IV.

‡Old male rats, week 22, receiving continuous basal diet.

§Large hyperplastic nodules isolated from liver of 6-week rats receiving basal diet after completing cycle 5 of the feeding regimen.
AST IV Expression in AAF Hepatocarcinogenesis

Fig. 2. A, autoradiogram and scan pattern of AST IV immunoprecipitated from in vitro translation products generated by hepatic mRNA of rats undergoing AAF-induced hepatocarcinogenesis. The procedures used for AST IV isolation and SDS-PAGE are described in “Materials and Methods.” M, molecular weight markers; C, control lane; B, young basal rat; C1, rat receiving AAF for 3 weeks; C1R, rat at the end of 3 weeks of basal diet subsequent to an initial 3 weeks of AAF administration; C3, cycle 3 of the feeding regimen; C3R, rats receiving basal diet for 3 weeks subsequent to C3; C5, cycle 5 of the feeding regimen; C5R, 3-week basal diet subsequent to C5; N, hyperplastic nodules; F, female; OB, 22-week rat receiving continuous basal diet; IIT, total in vitro translation products. Ordinate, molecular weight standards in thousands (kD); arrow, AST IV bands (M, 32,000–33,500 range). B, pattern of in vitro expression of AST IV by hepatic mRNA of rats during AAF hepatocarcinogenesis. Each point is a value of radioactivity in AST IV specific band relative to that in an equivalent negative control band region. Data were generated by radioanalytical image analysis of A. The hepatic mRNA of female rats translated no detectable levels of AST IV.

DISCUSSION

Extensive biochemical studies have shown that the administration of AAF to male Sprague-Dawley rats in a modified Teebor-Becker (32) hepatocarcinogenesis model mediates a dramatic loss in cytosolic NOHAAF AST IV activity (17, 30). The pattern of loss in AST IV activity appears to be bimodal, involving an early phase during which loss in enzyme activity is reversible to normal levels after suspension of AAF treatment and a late phase following prolonged exposure to AAF, when}

and recovery (Fig. 4) similar to that expressed by the in vitro translation of hepatic mRNA (Fig. 3). The periods of lowered cytosolic albumin expression (weeks 3, 11, and 19) were followed by >100% recovery of normal levels in treated rats that were returned to basal diet (Fig. 4). Furthermore, old rats (22 weeks) exhibited an age-related decrease in cytosolic levels of albumin similar to that observed for in vitro translation activity. The AAF-mediated lowering of hepatic cytosol albumin levels following cycle 5 correlates with reports (38–40) showing reduced levels of albumin in chemically induced hyperplastic nodules and hepatomas.

Differential Expression of AST IV by Rat Hepatic mRNA Mediated by Age, Gender, and AAF Treatment. It is known that female Sprague-Dawley rats express little or no hepatic NOHAAF sulfotransferase activity (17, 18) and are also less susceptible to AAF-induced hepatocarcinogenesis (15). It was therefore of interest to determine the capacity of female hepatic poly(A) mRNA to express AST IV in vitro. No detectable level of AST IV was determined in the translation product generated by female mRNA, although the levels of translation activity and expression of albumin were comparable to those of their male counterparts (Table 1). When young (week 0) and old (week 22) control rats were compared, a slight decrease was observed in the translation activity and expression of albumin (16 and 24%, respectively) by hepatic mRNA from old rats (Fig. 1; Table 1). The mRNA extracted from hyperplastic nodules showed both the levels of AST IV and albumin expression in vitro that were lowered to about 50 and 80%, respectively, of age-matched (week 25) control rats (Table 1).
there is irreversible loss of enzyme activity. The first phase also coincides with the stage in chemical hepatocarcinogenesis characterized by the evolution of morphologically distinct small hyperplastic nodules, with the nodules regressing after withdrawal of carcinogen treatment (32). The second phase is characterized by the emergence of hyperplastic nodules which persist even after withdrawal of carcinogen treatment and concomitant higher incidence of hepatoma formation (32, 41; reviewed in 29).

In the present study, our results showed that when rats are given AAF in their diet for 3 weeks, there was about a 50% decrease in the overall capacity of hepatic poly(A)+ mRNA for in vitro translation. The depressed overall translation activity was further accompanied by another 50% reduction in the specific expression of AST IV, to yield a cumulative reduction of about 4-fold (75%). This value for decreased AST IV expression corresponds well with equivalent 80–85% AAF-mediated lowering of cytosolic AST IV activity, reported in an earlier study (17, 30). Interestingly, the early stages of the carcinogenesis process were characterized by recovery of overall translation activity and albumin expression (100%) as well as AST IV (80%) expression to normal levels by mRNA of rats recovering from cycle 1 of the feeding regimen. The similarities in the pattern of depression and recovery of overall translation activity and of albumin and AST IV expression during the first cycle of the feeding regimen strongly suggest that AAF mediates a direct effect on total hepatic mRNA population, thereby inducing a general inhibitory effect on the expression of several hepatic proteins, but normal activity is recovered when exposure to the carcinogen is suspended. A mechanism for this lowering of the capacity of hepatic mRNA for overall translation activity may be the formation of AAF-RNA adducts by the highly reactive metabolite produced in the AST metabolic sulfation pathway. This may thus account for the initial 2-fold loss in the capacity of mRNA to express AST IV.

Adduct formation is known to be an early event in AAF-induced rat hepatocarcinogenesis (42, 43), while earlier studies by Irving (6) showed that sulfation of NOHAAF is necessary for toxicity. In a study in which the specificity of AF and AAF adduct formation were compared, a marked difference was found between the ratios of AAF and AF adducts formed with either DNA or RNA; furthermore, AAF was bound predominately to RNA while AF was more frequently bound to DNA (12). In another study reported by Meerman et al. (13), rats pretreated with pentachlorophenol (AST inhibitor) and later given NOHAAF were found to have a greatly decreased amount of fluorene covalently bound to hepatic RNA, but only a small decrease in the amount of fluorene bound to DNA, 4 h after carcinogen treatment. Accordingly, the further 2-fold loss in the capacity for specific expression of AST IV may be a consequence of relatively high concentrations of the activated metabolites generated in the vicinity of AST IV mRNA, thus increasing the levels of adduct formation with this mRNA species. Since DNA adducts are also generated in the carcinogenic process, the genetic lesions affecting transcription may also be induced. However, at the early stages of the hepatocarcinogenic process, the effects of such lesions would not be expected to be evident; hence there is ready resumption of the capacities of mRNA for overall translation and AST IV and albumin expressions when AAF treatment is suspended. In addition, it has been estimated that the turnover rate of mammalian mRNA is about 10 h (t₀) (44, 45); therefore toxic lesions on cytoplasmic RNA would be expected to be immediately reflected in altered translation activity, which appears to be the case during the 3 weeks of AAF administration with the feeding regimen used in the present study. Suspension of AAF treatment, however, provides over 500 h (3 weeks) available for the cytoplasmic RNA pool to be replenished to normal levels.

In contrast to the reversible nature of translation events observed initially during cycles 1 and 3 of the feeding regimen, at cycle 5 AAF mediates loss of AST IV expression by hepatic mRNA that is not reversible when the carcinogen treatment was suspended. This is also in contrast to the pattern observed for the capacity for overall translation and specific expression of albumin which were both recovered to normal levels (100%). The irreversible loss of AST IV expression correlates with the irreversible loss of enzyme activity observed previously in equivalent tissues (30). The mRNA isolated from hyperplastic nodules had overall translation capacity similar to that expressed by mRNA of 22-week age-matched control rats as well as hepatic mRNA of rats that were given basal diet for 3 weeks subsequent to completion of cycle 5 of the feeding regimen. The observed capacity of post-cycle 5 and nodular mRNA for albumin expression is in agreement with studies which have found unchanged or only slightly reduced levels of albumin in small hyperplastic foci, but significantly and variably reduced levels (10–90%) in large hyperplastic nodules and hepatomas (38, 40), when compared to normal control hepatocytes. Thus, to fully characterize the molecular mechanisms associated with the pattern of AST IV expression at the late stages of hepatocarcinogenesis, molecular regulation of the gene expression, qualitative and quantitative assessment of AST IV mRNA species, and gene sequencing to possibly localize AAF-induced mutations in the genomic coding sequence will be required. This is the focus of ongoing research efforts in our laboratory.

Generally, the results of the present study on the pattern of AST IV expression show a strong correlation between differential cytosolic expression in vivo and modulation of in vitro expression by rat hepatic poly(A)+ mRNA, during AAF-induced rat hepatocarcinogenesis. It is expected that detailed determination of the molecular events associated with the expression of AST IV during AAF-induced hepatocarcinogenesis will help, both in classifying the carcinogenic process into distinct biochemical steps as well as in obtaining a meaningful perspective on the role of its unique biochemical functions in the carcinogenic process.
ACKNOWLEDGMENTS

The assistance of Laura Smith in the preparation of the manuscript is gratefully acknowledged.

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Cancer Res 1991;51:504-509.