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DIVERSITY IN RAT STRAINS AND TUMOR LINES OF DNA FRAGMENTS HOMOLOGOUS TO AN AMPLIFIED 5.8 KILOBASE ECO R1 FRAGMENT OF NOVIKOFF HEPATOMA CELLS

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SUMMARY. The nuclear DNA of several different rat strains and rat tumor lines have been analyzed with respect to Eco R1 fragments homologous to the amplified 5.8 kb Eco R1 fragment (fragment A) of Novikoff hepatoma cells. Two Eco R1 fragments, 4.8 and 4.4 kb, which hybridized to the 5.8 kb Eco R1 fragment, were found in all the genomes investigated. Although none of the examined genomes exhibited evidence of the same degree of amplification of fragment A related sequences as that of Novikoff hepatoma, several had Eco R1 fragments of various other sizes which were homologous to fragment A. These results indicate that the family of fragment A homologous sequences consists of two populations, the constant 4.8 and 4.4 kb fragments, and a second group of sequences which varies with respect to size.

Novikoff hepatoma cells contain a DNA sequence which, when cleaved by Eco R1, yields a 5.8 kilobase pair (kb) fragment (fragment A) (1). Cloning and hybridization studies with this fragment demonstrated it to be amplified forty-fold in Novikoff hepatoma cells, relative to homologous sequences in normal rat liver (2). Southern hybridization studies of Eco R1 digests of Novikoff hepatoma and normal rat liver DNA (Sprague-Dawley ARS) demonstrated that two other Eco R1 fragments of 4.8 and 4.4 kb designated A' and A'', respectively, also hybridized to cloned fragment A. These observations suggested that fragment A might have resulted from a rearrangement and amplification of sequences found within the 4.8 kb and 4.4 kb fragments. It was of interest to examine the nature of fragment A related DNA sequences in a number of rat strains and in other rat tumor lines to ascertain if other examples of amplification of these sequences exist.

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In the present study, we examined the occurrence as well as sizes of Eco Rl produced DNA fragments homologous to fragment A in several different rat strains and transplantable hepatocellular carcinomas. All of the seven examined DNA samples exhibited the Eco Rl products, fragments A' and A''. In addition, several had Eco Rl fragments of other sizes which were homologous to fragment A. However, none of the examined genomes contained the same degree of amplification of fragment A related sequences as did that of Novikoff hepatoma.

These data demonstrate that rat fragment A genes represent a special familial gene set where diversity in the gene family is superimposed upon a set of conserved sequences. In previously reported examples of familial genes, the entire gene family has been found within genomes of single individuals (3-6). In the rat, the fragment A gene family is represented by a conserved subset of two fragments, A' and A'', present in all rat genomes thus far investigated, as well as a subset of fragments with varied representation.

MATERIALS AND METHODS

Animals and Tumor Lines. Rat strains used in these studies were Sprague-Dawley, ARS, two Sprague-Dawley CFE strains obtained from different commercial breeders, and strain ACI. Novikoff hepatoma was carried as an ascites tumor; Morris hepatoma 7777 (7,8) and hepatoma 252 were carried as solid tumors (9).

Isolation of DNA, Restriction Endonuclease Digestion and Southern Hybridization. DNA isolation, restriction endonuclease digestion, and agarose gel electrophoresis were carried out as previously described (1). Molecular weight standards, Eco Rl and Hind III digests of λ, were electrophoresed alongside experimental samples. Restriction endonucleases were routinely used as recommended by the suppliers at two units per μg DNA. Southern transfers and hybridizations were done as previously described (1) utilizing nitrocellulose paper. Following hybridization in buffered 3 x SSC (1XSSC is 0.15 M NaCl 0.015 M Na citrate, pH 7.0) at 68°C for 16-18 hr, filters were washed for 1 hour in 3 x SSC, 1 hour in 1 x SSC at 68°C and then dried and autoradiographed. Recombinant DNA, cloned in λgt-WES, was isolated as described (11) and nick translated (12).

RESULTS

As shown in Figure 1, all the nuclear DNA's analyzed had very similar Eco Rl digestion patterns. Major visible ethidium bromide stained bands

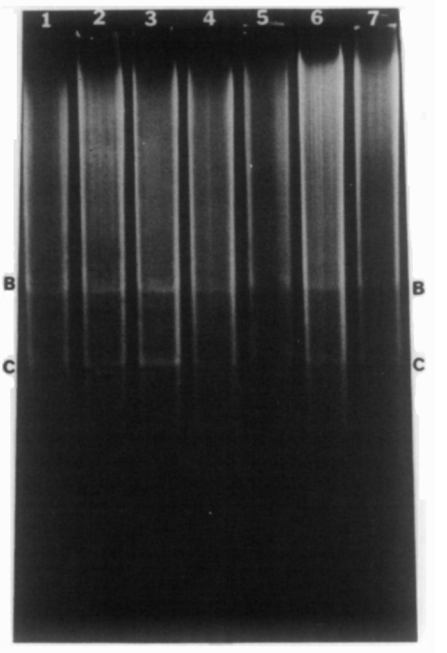

Figure 1. Photograph of Eco R1 digested nuclear DNA's of various rat strains, Novikoff hepatoma and transplantable rat hepatocellular carcinomas. Ten micrograms of each DNA was digested and electrophoresed as described in Materials and Methods. Lanes 1-4 contain DNA from Sprague-Dawley ARS (lane 1), Sprague-Dawley CFE (lane 2), ACI rats (lane 3) and Sprague-Dawley CFE from a second breeder (lane 4). Lanes 5-7 contain DNA from Novikoff hepatoma ascites cells (lane 5), hepatoma 7777 (lane 6), and hepatoma 252 (lane 7).

were bands B and C previously described by Parker et al. (2) which have also been described as bands A and B by Lapeyre and Becker (13). The 5.8 kb R1 fragment (fragment A) has not usually been visible in nuclear DNA digests (13-15). The digests shown in Fig. 1 were then transferred to nitrocellulose, hybridized to nick translated cloned fragment A and examined by autoradiography.

Each DNA examined contained 4.8 and 4.4 kb fragments (Fig. 2, arrows) which hybridized to fragment A. One of the Sprague-Dawley CFE strains (Fig. 2, lane 2) contained a 5.8 kb fragment, possibly homologous to fragment A itself, but not amplified; while the second CFE strain (Fig. 2, lane 4) contained no apparent additional homologous fragments. An additional band of 7.2 kb was found in strain ACI (lane 3), and both hepatoma 7777 (Fig. 2, lane 6) and 252 (Fig. 2, lane 7) contained additional hybridizable sequences

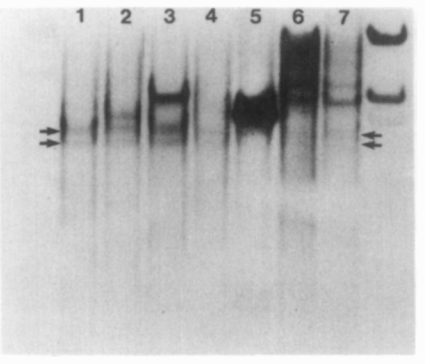

Figure 2. Autoradiography of various Eco R1 digested nuclear DNA's hybridized to cloned fragment A. The gel shown in Figure 1 was transferred and hybridized as described in Materials and Methods. The two outside lanes contain restriction endonuclease digests of λ which served as molecular weight markers.

of 7.2 and 9.4 kb. Such findings could represent the result of incomplete Eco R1 digestions. However, control experiments, in which enzyme to substrate ratio was tripled and time of digestion doubled, indicated that the results reflected total DNA digestion. The relative intensities of the hybrids indicated that the 5.8 kb fragment of Novikoff hepatoma was the only fragment exhibiting extensive amplification.

DISCUSSION

We have previously described the presence of a 5.8 kilobase pair Eco R1 fragment, fragment A, in the Novikoff hepatoma cell genome (1,2). Those studies demonstrated this fragment to be amplified approximately forty-fold relative to the number of copies of homologous sequences present in the genome of rat strain ARS. At least some of these homologous sequences were detected by Southern hybridization analysis, fragments A' and A''. In the present study, we have detected fragments A' and A'' in the genomes of two additional inbred rat strains and in two carcinogen-induced rat transplantable hepatocarcinomas. Several other high molecular weight Eco R1 fragments, which contain sequences homologous to fragment A, were also found in some of the genomes examined in this study.

Several possible mechanisms exist that might account for the heterogeneity of fragment A homologous DNA sequences reported here. DNA modification, e.g., methylation, can affect the susceptibility of DNA to cleavage by restriction endonucleases. If the methylation of the restriction enzyme sites which border the fragment A homologues were itself to be heterogeneous, several fragments of different sizes, which hybridize to fragment A, would be generated. Point mutations in some of the approximately 200 copies of the fragment A homologues would yield the same result as differences in DNA modification. Lastly, these findings may reflect different DNA fragments.

Homologous, but different, DNA fragments have previously been reported to exist due to variations in related genes. The ribosomal DNA's of Xenopus (3), Drosophila (4), mouse and man (6) are heterogeneous in size. Xenopus vitellogenin is apparently encoded by a family of related genes (5) composed of two divergent populations, each of which is composed of two more populations.

The heterogeneity of the fragment A homologues within each of the populations studied may be due to a related phenomenon. However, the heterogeneous distribution of the 5.8, 7.2 and 9.4 kb Eco R1 fragments, which are homologues to fragment A, may be due to specific selective pressures or a result of neutral drift between populations. Most particularly, the heterogeneity found within separately outbred colonies of CFE rats indicates that the variations reported here can arise due to genetic drift over relatively short periods of time. These data may also reflect the consequence of transposable genetic elements in a higher eukaryote. However, the present data, of themselves, do not constitute a rigorous proof of a eukaryotic transposon.

Cloning of the fragment A homologues and comparative sequence studies should provide a better understanding of their relationships to one another, as well as providing an assessment of just how far these sequences have drifted.

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