Chretien, M. (1981) *J. Biol. Chem. 256*, 7977–7984.
Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5205.
van der Rest, M., & Fietzek, P. (1982) *Eur. J. Biochem. 124*, 491–496.
van der Rest, M., Mayne, R., Ninomiya, Y., Seidah, N. G., Chretien, M., & Olsen, B. R. (1985) *J. Biol. Chem. 260*, 220–225.
Vaughan, L., Winterhalter, K. H., & Bruckner, P. (1985) *J. Biol. Chem. 260*, 4758–4763.

Amplified DNA of the Novikoff Hepatoma Nucleolus Is Arranged in a 7.3-Kilobase Tandem Repeat†

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ABSTRACT: We have reported previously the cloning and characterization of a nucleolar-localized 5.8-kilobase (kb) *Eco*RI fragment that is approximately 50-fold more highly reiterated in Novikoff hepatoma tumor cells than in normal rat liver [Parker, D. L., Busch, H., & Rothblum, L. I. (1981) *Biochemistry 20*, 762]. In the present study, the arrangement of these 5.8-kb *Eco*RI segments within the Novikoff hepatoma genome was investigated. Through the use of "indirect" restriction site mapping, partial restriction enzyme digestions, and molecular cloning, we have determined that the 5.8-kb *Eco*RI fragment and a 1.5-kb *Eco*RI fragment together constitute a 7.3-kb unit. The 7.3-kb unit is present in the hepatoma genome as a tandem repeat and constitutes the unit of the DNA that has been amplified. Studies on the arrangement of homologous sequences in the normal rat genome indicate that the amplified DNA may have been derived by a rearrangement and amplification of the nontranscribed spacer of the ribosomal DNA (rDNA) repeat.

Novikoff hepatoma cells have been shown to contain a moderately repetitive (4000–10 000 copies per haploid genome), 5.8-kilobase (kb) *Eco*RI fragment that is nucleolar localized yet does not hybridize to ribosomal RNA (rRNA) (Parker et al., 1981). Reassociation studies, using a cloned version of the 5.8-kb fragment as probe, demonstrated that these sequences were approximately 50-fold more abundant in Novikoff hepatoma cells than in normal rat liver DNA. Comparative Southern blots of *Eco*RI-digested normal rat liver and Novikoff hepatoma DNA failed to reveal the presence of a homologous 5.8-kb fragment in the liver. Two less intense hybrids of lower molecular weight (4.5 and 4.1 kb) were detected in roughly equal intensities in both the tumor and nontumor DNAs (Rothblum et al., 1982). The relative abundance of the 5.8-kb fragment, and its specific subnuclear localization (nucleolus), suggested that it may have evolved through the rearrangement and amplification of a piece of nucleolar DNA.

Amplified DNA segments have generally been found to exist in clusters [Schmike et al., 1978; Miller et al., 1979; reviewed by Schimke (1984) and by Stark & Wahl (1984)], and within a given cell population there exist subpopulations with varying degrees of multiplicity of specific genes (Wahl et al., 1979b; Alt et al., 1976). In many of the examined cases, with the exception of the amplified ribosomal DNA (rDNA) genes described by Miller et al. (1979) and the chorion protein genes (Spradling & Mahowald, 1980), the cells or cell lines that contain the amplified genes have been selected for by multiple rounds of exposure to a specific metabolic inhibitor. As a consequence of these manipulations, the amplified genes contain several different "novel joints", which may be the products of several rounds of amplification and recombination (Caizzi & Bostock, 1982; Schimke, 1984).

The examination of the genomic arrangement of the 5.8-kb fragment within the hepatoma genome was undertaken to determine the structure of the amplified DNA. We have also attempted to isolate the homologue of the 5.8-kb fragment in the normal rat genome. Several clones from the normal rat genome were isolated that very closely matched the restriction enzyme digestion pattern of the amplified fragment. Some of these clones represent the ribosomal nontranscribed spacer.

Materials and Methods

DNA Isolation and Restriction Analysis. All DNA isolations were performed as described previously (Parker et al., 1979). Restriction enzyme digestions were performed as recommended by the suppliers.

Partial restriction enzyme digestions were performed by the addition of 1 unit of enzyme/5 μg of DNA and aliquots removed at 3-min intervals. Southern transfers and hybridizations were performed as described by Wahl et al. (1979a).

^{32}P Labeling and Cloning of DNA Fragments. All radiolabeled DNA fragments were prepared by "nick translation" (Mackey et al., 1977). Cloning was performed by using the gt-WES system provided to us by Dr. P. Leder (Tiemeier et al., 1979) or by using a partial *Eco*RI rat genome library cloned in Charon 4A (generously provided by Drs. Tom Sargent and J. Bonner and their colleagues). Colony screening was performed essentially as described by Benton & Davis (1977). In some screenings, the stringency was increased by carrying out the final washes at 65 °C and in 0.1 × standard

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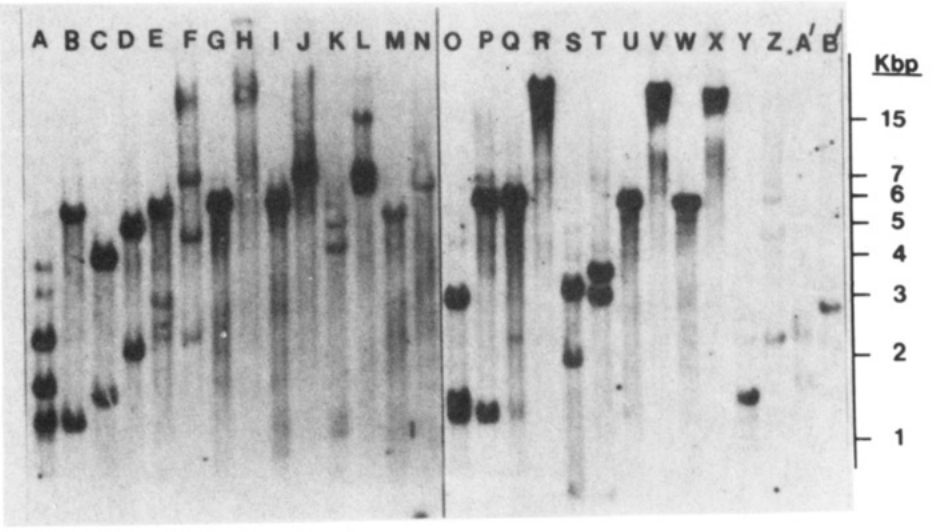

FIGURE 1: Southern blot hybridization of ^{32}P-labeled cloned 5.8-kb *Eco*RI fragment to Novikoff hepatoma nucleolar DNA, after restriction endonuclease digestion as follows: (A) *Xba*I/*Eco*RI; (B) *Xba*I; (C) *Hin*cII/*Eco*RI; (D) *Hin*cII; (E) *Hae*II/*Eco*RI; (F) *Hae*II; (G) *Kpn*I/*Eco*RI; (H) *Kpn*I; (I) *Sma*I/*Eco*RI; (J) *Sma*I; (K) *Xho*I/*Eco*RI; (L) *Xho*I; (M) *Pst*I/*Eco*RI; (N) *Pst*I; (O) *Sst*I/*Eco*RI; (P) *Sst*I; (Q) *Sal*I/*Eco*RI; (R) *Sal*I; (S) *Bgl*II/*Eco*RI; (T) *Bgl*II; (U) *Bgl*I/*Eco*RI; (V) *Bgl*I; (W) *Hpa*I/*Eco*RI; (X) *Hpa*I; (Y) *Bam*HI/*Eco*RI; (Z) *Bam*HI; (A′) *Hin*dIII/*Eco*RI; (B′) *Hin*dIII.

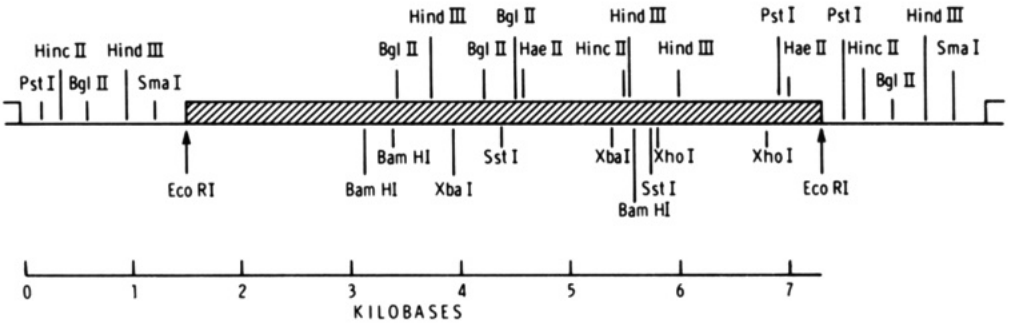

FIGURE 2: An extended restriction enzyme map of the tumor-amplified nucleolar DNA fragment. The map was derived in part from the data shown in Figure 1 and from double digestions with *Sma*I and *Pst*I as the second enzymes.

saline citrate solution (SSC). DNA sequencing was performed as described by Maxam & Gilbert (1977).

RESULTS

Structure of the Amplified Fragment in the Novikoff Hepatoma. The restriction enzyme map of the cloned 5.8-kb *Eco*RI fragment has been reported previously (Parker et al., 1981). Those regions of the Novikoff hepatoma genome located immediately adjacent to the nucleolar 5.8-kb *Eco*RI fragment were restriction site mapped as follows. Novikoff hepatoma nucleolar DNA was first digested to completion with the enzyme whose cleavage site was to be determined, an aliquot was removed, and the remaining portion was redigested with *Eco*RI. These single- and double-digested DNA samples were electrophoresed in agarose, blotted onto nitrocellulose, and hybridized with the cloned 5.8-kb probe, which had been ^{32}P labeled by nick translation.

The resulting autoradiograph (Figure 1) was then analyzed to accurately determine the size of those fragments that hybridized to the radiolabeled probe. The placement of individual restriction sites was determined by comparing the fragment sizes produced by each enzyme to the restriction map of the cloned 5.8-kb *Eco*RI fragment (Parker et al., 1981). The secondary *Eco*RI digestions provided a common reference point on the existing restriction map. The accuracy of the placement of these sites was confirmed by performing similar double-digestion series using *Pst*I and *Sma*I as the secondary enzymes (data not shown). The "extended" restriction map that was obtained is shown in Figure 2.

The resulting restriction map indicated that the central 5.8-kb *Eco*RI fragment was bordered on both sides by similar 1.5-kb *Eco*RI fragments, suggesting that the 5.8-kb *Eco*RI fragment was a portion of a larger, 7.3-kb tandemly repeating structure.

To test this hypothesis, partial restriction enzyme digestions of Novikoff nucleolar DNA were performed, using two of the mapping enzymes, *Eco*RI and *Sma*I, independently. The partial cleavage products were separated by agarose gel electrophoresis, blotted to nitrocellulose, and hybridized with radiolabeled 5.8-kb cloned probe. Fragments homologous to the 5.8-kb probe were visualized by autoradiography (Figure 3A). As predicted from the restriction map in Figure 2, the *Sma*I limit digestion product was 7.3 kb (Figure 3A, lanes S), with a "ladder" of partial digestion products increasing in 7.3-kb increments. The products of partial digestion by *Eco*RI are slightly more complex. The presence of two *Eco*RI sites per 7.3-kb repeat results in a ladder consisting of clusters of three. The total *Eco*RI digestion product of 5.8 kb was followed by fragments at 7.3 and 8.8 kb, representing the sequential inclusions of the flanking 1.5-kb *Eco*RI segments. The next set of three fragments were 13.1, 14.6, and 16.1 kb; each set was displaced from the prior set by 7.3 kb.

At least four contiguous 7.3-kb repeat units were visualized by this approach. A schematic representation of the repeating structure is depicted in Figure 3B. Verification of the structure and restriction map of the full 7.3-kb repeat was obtained by

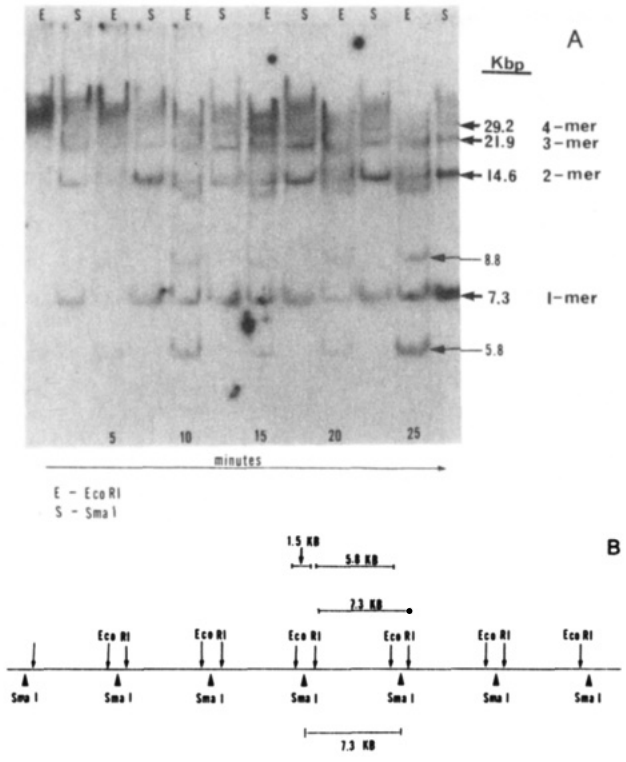

FIGURE 3: The amplified 5.8-kb *Eco*RI fragment is part of a tandemly repeating 7.3-kb unit. (A) Autoradiograph of a Southern blot of Novikoff hepatoma nucleolar DNA that was digested with either *Eco*RI (E) or *Sma*I (S) at an enzyme concentration of 1 unit/5 μg of DNA for the indicated times, electrophoresed in agarose, transferred to nitrocellulose, and hybridized with ^{32}P-labeled cloned 5.8-kb *Eco*RI fragment. (B) Schematic representation of the proposed 7.3-kb repeating structure of the tumor-amplified, nucleolar DNA fragment.

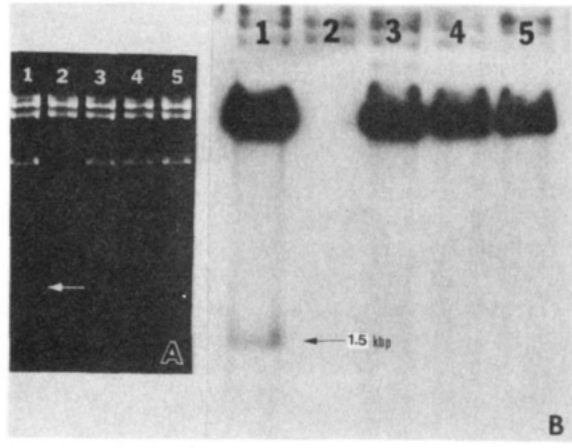

FIGURE 4: Partial characterization of a clone containing the 7.3-kb repeat. (A) Ethidium bromide stained agarose gel depicting the positive 7.3-kb clone in lane 1 (arrow points to 1.5-kb fragment). Lanes 3–5 are clones that contain only the 5.8-kb fragment, and lane 2 is a negative clone. (B) Southern blot autoradiograph of (A) hybridized to ^{32}P-labeled, cloned 5.8-kb *Eco*RI fragment. The arrow points to the 1.5-kb segment, which cross-hybridizes to a small extent with the 5.8-kb segment.

cloning partial *Eco*RI-digested Novikoff nucleolar DNA with use of the gt-WES system. Several positive clones were identified, one of which (Figure 4A,B) was restriction enzyme mapped and was identical with the map in Figure 3 (data not shown).

Structure of Homologues of the 5.8-kb Fragment in the Normal Rat Genome. Previous studies on the structure of fragments homologous to the amplified fragment had demonstrated that two *Eco*RI fragments, 4.5 and 4.1 kb, were

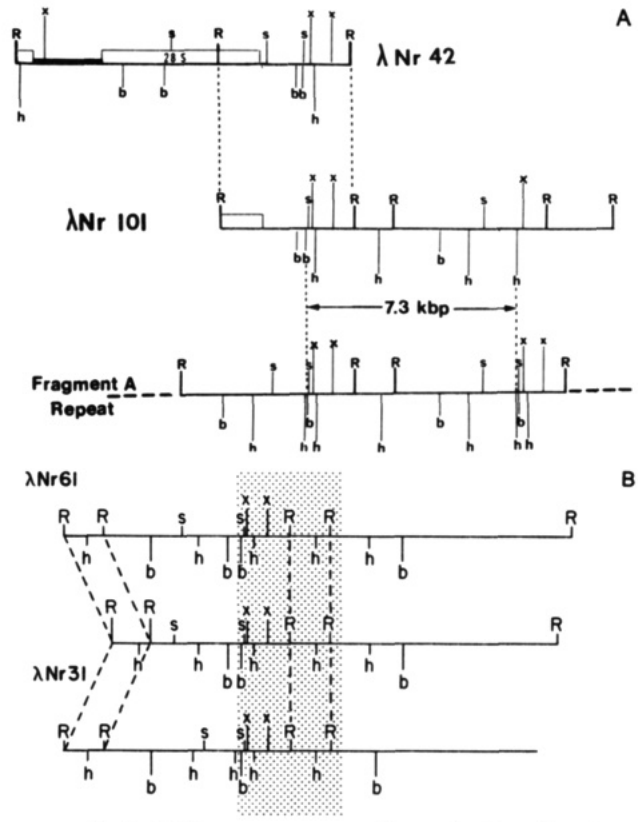

FIGURE 5: Restriction enzyme maps of genomic clones homologous to the 5.8-kb amplified *Eco*RI fragment. (A) Two clones of ribosomal DNA. The rDNA region that presumably gave rise to the 7.3-kb repeat is indicated. (B) Two nonribosomal clones that were selected by hybridization to the 5.8-kb *Eco*RI fragment. The region of restriction endonuclease homology is stippled. Symbols: R, *Eco*RI; x, *Xho*I; b, *Bam*HI; s, *Sst*I; h, *Hin*dIII.

present in the genomes of each rat strain or cell line investigated (Rothblum et al., 1981). This suggested that these fragments may have been the source of the DNA that had been amplified and that an investigation of the structure of these fragments might yield information pertinent to the understanding of the derivation of the 7.3-kb repeating structure. A rat genomic library consisting of partial *Eco*RI fragments was screened under stringent conditions (0.1 × SSC, 65 °C) with the 5.8-kb *Eco*RI fragment, which had been subcloned into the *Eco*RI site of pBR322. Positive plaques were purified and restriction enzyme mapped, and the regions homologous to the 5.8-kb fragment were determined by Southern hybridization experiments. The results that were obtained for four of the clones selected in this way are presented in Figure 5. Both Nr 42a and Nr 101 (Figure 5A) were found to contain 4.5-kb *Eco*RI fragments that hybridized to the 5.8-kb *Eco*RI fragment. The restriction map of Nr 42a was identical with that of Nr 42 (Rothblum et al., 1982), and it was determined that Nr 42a did in fact contain the 6.6-kb *Eco*RI fragment (18S–28S portion) of the rat ribosomal RNA transcription unit. Further, the 4.5-kb *Eco*RI fragment that hybridized to the Novikoff hepatoma 5.8-kb *Eco*RI fragment contained the 3′ terminus of 28S rRNA (data not shown). Nr 101 also contained the 3′ terminus of 28S rRNA in a 4.5-kb *Eco*RI fragment as well as several other *Eco*RI fragments, including a 1.5-kb fragment, indicating that this clone extended further into the nontranscribed spacer region.

We were able to more fully examine the apparent relationship between Nr 42 and the 5.8-kb fragment of the Novikoff hepatoma by comparing part of the sequence of the 3′ terminus of the 4.5-kb *Eco*RI fragment of Nr 42 (Mroczka

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1. CTTGTGCTTC CTAGGCAAGC GCTCTACCAC TGAGCTAAAT CCCCAACCCC GAATGTTTAA
2.                                                  ********** N**********

1. TGTTTAATGT TTAAATAAAT AAACCTTGTA AAGAAATT-A AGCATCTTAA AACCATAGGC
2. ********** ********** ********** *T*******T* ********** **********

1. ATGTGCATCC CCTCCTCATT CGTTCATTCC CTCGCTCATT CATTCATTCA TTCATTCAGA
2. ********** ********** ********** ********** ********** **********

1. CAGACAGACA GACAGACAGA CAG------CA ACCAAAGTCT AGCCTTTAAC AAGCAAGAAA
2. ********** ********** ***ACAGA** ********** ********** **********

1. GCATC
2. *****
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FIGURE 6: Sequence homology between *Eco*RI fragment A and rat rDNA. Comparison of a portion of the published sequence (Mroczka et al., 1984) of the rat ribosomal DNA nontranscribed spacer (line 1) with that of the partial sequence of the Novikoff hepatoma *Eco*RI fragment (line 2) (Parker et al., 1981). The sequences have been aligned to maximize homology. An asterisk indicates homology, and a dash or N indicates an insertion.

et al., 1984) to that of the corresponding region of the 5.8-kb *Eco*RI fragment (Parker et al., 1981) (Figure 6). Both sequences contained arrays of repeating nucleotides and overall were nearly exact duplicates of one another (94% homology). The sequence data and the restriction maps suggested that the amplified rDNA region extended from near the *Bam*HI site to the *Hin*dIII site (the area within the dashed lines, Figure 5).

The restriction enzyme maps of the transcribed portions of the rDNA of the Novikoff hepatoma and normal rat liver are essentially identical (Parker and Rothblum, unpublished observations). Thus, the data would indicate that the 7.3-kb repeat has arisen from an amplification and rearrangement of the rDNA repeat that did not involve the transcribed region.

The arrangements of the restriction endonuclease sites in two other characterized clones, Nr 61 and Nr 31, were also highly homologous to those of the fragment A clone as well as to one another (Figure 5B). Nr 61 and Nr 31 were very similar, except for an apparent insertion in the "5.8-kb *Eco*RI fragment" of Nr 61 and a deletion in that fragment in Nr 31. Both clones contained 1.5-kb *Eco*RI fragments flanking the "5.8-kb" core fragment.

When the restriction enzyme maps of Nr 101, Nr 31, Nr 61, and fragment A were compared, several common elements became apparent. They each contained almost exact duplicates of a "core" region (stippled regions indicated in Figure 5B) of the rDNA repeat. All of the clones contained a 1.5-kb *Eco*RI fragment, and in both Nr 31 and Nr 61 two copies of the 1.5-kb *Eco*RI fragment flank the region homologous to the rDNA core.

Discussion

The present study was undertaken in order to better define the genomic arrangement of a 5.8-kb *Eco*RI fragment amplified in the Novikoff hepatoma cell. By determining restriction enzyme cleavage sites adjacent to the genomic 5.8-kb *Eco*RI fragment, we have demonstrated that the fragment is flanked by identical 1.5-kb *Eco*RI fragments. The hypothesis that the 5.8-kb fragment was part of a 7.3-kb tandemly repeating structure was confirmed by partial restriction endonuclease digestion and Southern blot hybridizations. A full-length, 7.3-kb repeating unit was obtained by molecular cloning; the restriction map of this clone agreed with that predicted by the initial genome blot experiments using genomic DNA.

When the amplified fragment of Novikoff hepatoma cells was used to select clones from the normal rat genome, two of the clones selected were portions of the rat ribosomal DNA repeat. In itself this was interesting as the 5.8-kb *Eco*RI fragment is a nucleolar fragment. Although the amplified DNA may not be part of the rDNA repeat in the Novikoff hepatoma, it was felt that the fragment might have a role in nucleolar function. Analysis of the restriction maps of Nr 101 and Nr 42a indicated that the 7.3-kb repeat may be directly derived from the nontranscribed spacer (NTS) of the rDNA repeat and that the amplification event generated apparently identical tandem copies of the 7.3-kb repeat unit. This would indicate that there may have been a single amplification event and suggests that it may be possible to identify the sites (novel joints) at which the 7.3-kb repeat unit is inserted in the Novikoff genome by walking the amplified region. It will be of interest to determine if the 7.3-kb repeats are uniformly distributed in the NTS of the ribosomal genes of the Novikoff hepatoma or if they have segregated. In this regard, it should be noted that as the Novikoff hepatoma is a heteroploid cell line, there may be variants in the structure of the amplified fragment that would not be detectable against the general pattern.

The genomic clones Nr 61 and Nr 31 contain regions of homology to the 7.3-kb repeat that are homologous to the rDNA NTS, as well as flanking copies of the 1.5-kb *Eco*RI fragment. However, in these clones the rDNA core region is contiguous with sequences that have apparently become associated with this DNA through either a genomic rearrangement or recombination event. The finding that the 7.3-kb repeat may have arisen from the rDNA repeat is consistent with the original estimates of 200 copies of the precursor in the normal rat genome. However, the finding of nonribosomal DNA fragments homologous to the 7.3-kb repeat suggests that the original measurement of the copy number may have been an underestimate. A discussion of how the original estimates could have been in error is presented by Whitney & Furano (1984).

If in fact the amplified fragment found in the Novikoff cells did originate from the NTS of the rDNA, then the association of that DNA with other sequences may be analogous to the findings of Kominami et al. (1983), who have reported that a region of the 3′ NTS of mouse rDNA, the PR1 sequence, is found recombined with other sequences. They also reported that these recombinant sequences have been amplified to varying degrees in different mouse strains and cell lines.

Taken together, our studies and the results of Kominami et al. (1983) suggest that at least one region of the nontranscribed spacer is highly fluid, capable of extensive rearrangements and amplification. The significance of this amplified sequence is unknown. Work is continuing in order to further define the relationship of the homologous sequences in the normal rat liver genome. These results strongly suggest that sequences homologous to those found in the 7.3-kb sequence originated from within the nontranscribed spacer region

of rDNA. It is not known whether this fragment is retained within the nontranscribed spacer of the rDNA of Novikoff hepatoma cells or if it serves a function. It may represent fortuitously amplified sequences, "selfish DNA", with no particular function. Future studies on this fragment should provide insight into the mechanism and process of gene amplification.

REFERENCES

Alt, F. W., Kellems, R. E., & Schimke, R. T. (1976) *J. Biol. Chem. 251*, 3063.

Benton, W., & Davis, R. W. (1979) *Science (Washington, D.C.) 196*, 180.

Caizzi, R., & Bostock, C. (1982) *Nucleic Acids Res. 10*, 6587.

Jelinek, W. R., & Schmid, C. W. (1982) *Annu. Rev. Biochem. 51*, 813.

Kominami, R., Urano, Y., Mishima, Y., & Muramatsu, M. (1983) *J. Mol. Biol. 165*, 209.

Mackey, J. K., Brackman, K. H., Green, M. R., & Green, M. (1977) *Biochemistry 16*, 4478.

Maxam, A. M., & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 560.

Miller, O. J., Tantravanhi, R., Miller, D. A., Yu, L. C., Szabo, P., & Prensley, W. (1976) *Chromosoma 71*, 183.

Mroczka, D. H., Cassidy, B., Busch, H., & Rothblum, L. I. (1984) *J. Mol. Biol. 174*, 141.

Parker, D. L., Rothblum, L. I., & Busch, H. (1979) *Cancer Res. 39*, 1287.

Parker, D. L., Busch, H., & Rothblum, L. I. (1981) *Biochemistry 20*, 762.

Rothblum, L. I., Parker, D. L., Cassidy, B., Becker, F., Busch, H., & Rodriguez, L. (1981) *Biochem. Biophys. Res. Commun. 101*, 639.

Rothblum, L. I., Parker, D. L., & Cassidy, B. (1982) *Gene 17*, 75.

Schimke, R. T. (1984) *Cell (Cambridge, Mass.) 37*, 705.

Schimke, R. T., Alt, F. W., Kellems, R. E., Kaufman, R. J., & Bertino, J. R. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 649.

Spradling, A. C., & Mahowald, A. P. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1096.

Stark, G. R., & Wahl, G. M. (1984) *Annu. Rev. Biochem. 53*, 447.

Tiemeier, D. C., Tilghman, S. M., & Leder, P. (1977) *Gene 2*, 173.

Wahl, G. M., Stern, M., & Stark, G. R. (1979a) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3683.

Wahl, G. M., Padgett, R. A., & Stark, G. R. (1979b) *J. Biol. Chem. 254*, 8679.

Whitney, F. R., & Furano, A. V. (1984) *J. Biol. Chem. 259*, 10481.

External Calcium Inhibits the Efflux of Calcium from Isolated Retinal Rod Outer Segment Disks

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ABSTRACT: Increasing the concentration of calcium in the external buffer flowing past isolated, intact bovine retinal rod outer segment disks immobilized in a flow system reduced the rate of radioactive calcium efflux from within the disks in the dark. We interpret these results as extradiskal calcium acting at an inhibitory binding site to block the calcium efflux. A Scatchard analysis of the external calcium dependence of the efflux yields an apparent dissociation constant of 50 μM, which further suggests that the inhibition is mediated by a specific membrane binding site. The observed inhibition of calcium efflux may represent a functional role for the high-affinity calcium binding site which has been identified by others in previous physical studies of the disk membrane. This external calcium inhibited permeability may explain some of the discrepancies in the reported calcium transport properties of disks. Variations in the external calcium concentration may alter the calcium content of isolated disks, thereby indirectly affecting other transport functions including the measured light-induced release of calcium. No evidence was found for either Na/Ca or Ca/Ca exchange processes across the disk membrane. Lanthanum was even more effective than calcium in inhibiting calcium efflux in the dark. Neither lanthanum nor calcium inhibited the light-induced efflux of calcium from disks, which implies either that light and extradiskal calcium regulate separate permeability processes in the disk membrane or that light greatly reduces the affinity of the inhibitory site for calcium and lanthanum.

The fundamental light-transducing element in vision is rhodopsin, an integral membrane protein which, in retinal rod cells, is found in the intracellular disk organelles within the rod outer segment. In spite of intensive efforts, no process has been proven to be the physiological link between the light energy absorbed by rhodopsin in the disk membrane and the observed change in sodium permeability that hyperpolarizes the plasma membrane to initiate the neural signal which results in vision. Although conclusive proof is not available, substantial evidence has accumulated to support Hagins' hypothesis (Hagins, 1972) which suggests that, upon light exposure, the disks release calcium ions which diffuse to the plasma membrane where they block sodium channels.

This hypothesis is supported by the observed inhibition of the light-sensitive sodium conductance of the rod outer segment plasma membrane by extracellular calcium (Yoshikami & Hagins, 1973). Calcium ionophores increase the sensitivity of the sodium conductance to extracellular calcium, which

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