Vol. 114, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 29, 1983 Pages 670-676

THE MAJORITY OF cDNA CLONES WITH STRONG POSITIVE SIGNALS FOR THE INTERFERON-INDUCTION-SPECIFIC SEQUENCES RESEMBLE MITOCHONDRIAL RIBOSOMAL RNA GENES

Teruhisa Tsuzuki, Hisayuki Nomiyama, Chiaki Setoyama, Shuichiro Maeda, Kazunori Shimada, and Sidney Pestka*

Department of Biochemistry
Kumamoto University School of Medicine
Kumamoto 860, Japan

*Roche Institute of Molecular Biology
Roche Research Center
Nutley, NJ 07110 (U.S.A.)

Received June 17, 1983

SUMMARY: A complementary DNA library prepared from the 12S polyadenylated RNAs extracted from interferon-induced KG-1 cells, a human myeloblast cell line, was screened for the presence of induction-specific sequences. Clones that exhibited strong positive signals were separated by hybridization criteria into nine classes. Clones from classes I through IV consisted of about 78% of the total and unexpectedly were found to resemble human mitochondrial ribosomal RNA genes.

Maeda *et al.* (1) cloned DNAs complementary to the 12S polyadenylated RNAs extracted from human myeloblast cells induced to produce interferon. To isolate interferon cDNA clones, these clones were screened for the presence of DNAs complementary to the inducible mRNAs by colony hybridization with a mixture of ^{32}P-labeled 12S polyadenylated RNAs prepared from interferon-induced cells as a probe in the presence of unlabeled excess polyadenylated RNAs from uninduced cells. Positive clones were originally classified into two groups (1). One group exhibited dense spots and the second group yielded faint spots on autoradiography. Two interferon cDNA clones were isolated from the second group but none from the first group of colonies that exhibited intense spots.

To investigate whether these clones exhibiting dense spots contain DNAs complementary to highly abundant polyadenylated RNAs present in the interferon-induced cells, we initiated studies on these cDNA clones. The results of these studies are reported here.

Abbreviations: cDNA, complementary DNA; bp, base pairs; kb, kilobase pairs.

0006-291X/83 $1.50

MATERIALS AND METHODS

Enzymes and Chemicals. Restriction enzymes were purchased from Takara Shuzo Co. and were used according to the supplier's recommendations. *Escherichia coli* DNA polymerase I was obtained from BRL. DNase I was purchased from Sigma and [α-^{32}P]dCTP (3,000 Ci/mmol) was from Amersham.

DNA Isolation. Human genomic DNAs were prepared from human placenta as described by Blin and Stafford (2). Plasmid DNAs were prepared as described by Mukai *et al.* (3). Propagation of *E. coli* cells carrying recombinant plasmids was carried out in accordance with the guidelines for recombinant DNA research, issued by the Ministry of Education, Science and Culture of Japan as well as in accordance with the NIH Guidelines for Recombinant DNA Research.

DNA Blot Hybridization. Electrophoresis of DNAs was performed on 0.8% agarose horizontal slab gels containing 0.5 µg of ethidium bromide per ml (3). The molecular weights of the restriction fragments were estimated with reference to *Hind*III digests of lambda DNA. After electrophoresis, the digests of the DNAs were transferred to nitrocellulose filters by the method of Southern (4). In some experiments, duplicate DNA blots were prepared from a single agarose gel as described by Smith and Summers (5). The resulting blots were hybridized with appropriate ^{32}P-labeled probes. The hybridization was carried out in the presence of 10% dextran sulfate as described by Wahl *et al.* (6). The removal of the hybridized probe was accomplished by washing the filters in 0.05X wash buffer for 3 h at 65°C, following the procedure of Thomas (7). The 1X wash buffer contains 50 mM Tris·HCl, pH 8.0, 2 mM EDTA, 0.5% sodium pyrophosphate, and 0.02% each of bovine serum albumin, polyvinyl pyrrolidone, and Ficoll 400.

Nucleic Acid Labeling. Nick translations were performed according to the method of Rigby *et al.* (8). All the cDNA inserts used as probes for hybridization experiments were excised from the recombinant plasmid DNAs prior to labeling.

Gene Copy Number Estimation. The copy number of the human genomic DNA sequences hybridizing with the cDNA probes was estimated as described by Griffin-Shea *et al.* (9).

RESULTS AND DISCUSSION

Classification of cDNA Clones. The isolation of 60 cDNA clones exhibiting high intensity on autoradiography was described previously (1). Fifty eight out of the 60 clones carried DNA inserts larger than 200 bp and were chosen for the following studies. The classification of these clones were carried out by colony hybridization (10) with appropriate nick-translated cDNAs as probes. Twenty seven out of the 58 clones carried DNA sequences complementary to a cDNA insert excised from one of the clones designated pHIG-1, and 24 clones hybridized with another cDNA insert of another clone, pHIG-15. The former class is named class I and the latter, class II. Although the pHIG-15 cDNA did not hybridize with the pHIG-1 cDNA, 10 of class I and 10 of class II clones hybridized with each other. This common subset is considered class III and a representative of this class is pHIG-2. The remaining 17 clones were further

TABLE 1. CLASSIFICATION OF THE cDNA CLONES

Class	Plasmid[a]	Number of Colonies
I	pHIG-1	27 (17)[b]
II	pHIG-15	24 (14)[b]
III	pHIG-2	10[b]
IV	pHIG-34	4
V	pHIG-4	6
VI	pHIG-61	4
VII	pHIG-22	1
VIII	pHIG-35	1
IX	pHIG-57	1

[a]Designation of plasmid used as a representative of each class. The cDNA inserts derived from these plasmid DNAs were used as probes for classification. All cDNA clones within a class cross-hybridized with each other but not with members of the other classes, except for the following. Class III consists of a common subset of clones from classes I and II which hybridize with both class I and II cDNAs.

[b]Ten clones of class I and 10 of class II hybridized with each other and were classified as class III. Thus, only 17 and 14 cDNA clones were uniquely in classes I and II, respectively.

classified by repeating the same procedures. Finally, all the 58 clones were classified into one of 9 classes, as summarized in Table 1. The cDNA inserts isolated from class III clones hybridized with those from both classes I and II, whereas none from any other classes cross-hybridized with members of the other classes.

Southern Blot Analysis of the Human DNA Complementary to the cDNA Clones. The cDNA clones from class I through IV hybridized strongly with human placental DNA. By a reconstruction experiment (9), we estimated that the copy number of the genomic DNA corresponding to pHIG-1 is 20 to 30 per haploid human placental genome (data not shown). To analyze the structural features of the human DNA complementary to these cDNA clones, Southern blot analysis of the human DNA was carried out. At first, human placental DNA was digested with *Bgl*II, *Eco*RI, *Bam*HI, *Pst*I, and *Kpn*I, as well as paired combinations. The digests were then electrophoresed on 0.8% agarose gels and blotted onto nitrocellulose filters. The DNA blots were hybridized with the nick-translated pHIG-1 cDNA probe. As shown in Fig. 1A, their strong hybridization was detected on the autoradiogram. Its simple pattern and the very discrete bands suggest that most of the DNA sequences complementary to the pHIG-1 cDNA probe are tandemly repeated in the human genome or exist as a multicopy extra-chromosomal element.

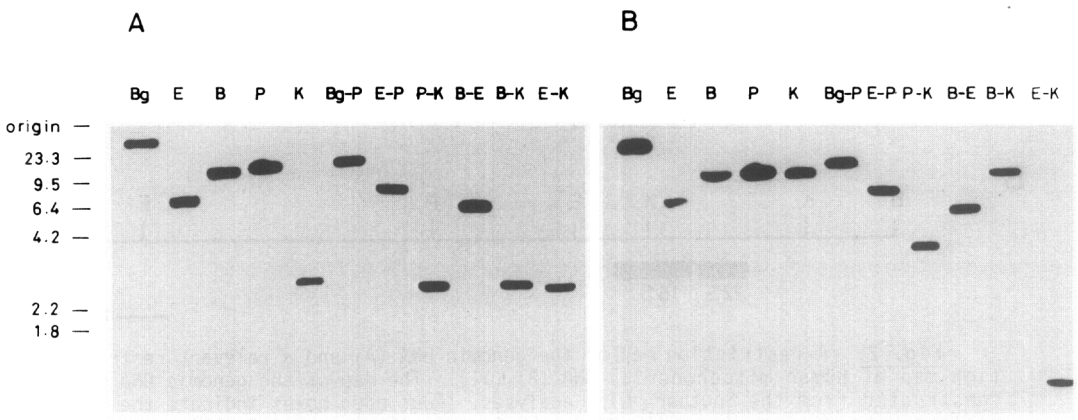

Fig. 1. Southern blot analysis of human DNA probed with several cDNA clones. Ten µg of human DNA cleaved with *Bgl*II (Bg), *Eco*RI (E), *Bam*HI (B), *Pst*I (P), and *Kpn*I (K) as well as with paired combinations of these were loaded onto 0.8% agarose gels as indicated. After electrophoresis, DNA was transferred bidirectionally onto two nitrocellulose filters (5). One of the duplicate blots was hybridized with ^{32}P-labeled pHIG-1 cDNA (A), and the other with ^{32}P-labeled pHIG-15 cDNA (B). Numbers on the left indicate the size in kb of DNA markers (lambda DNA cleaved with *Hind*III) on the same gel. The original gel before transfer was slightly distorted giving the false impression on this Southern blot that some fragments digested with two enzymes migrated slower than some digested with the individual enzymes.

Similar Southern blot analyses of the human placental DNA was performed with pHIG-15, pHIG-2, and pHIG-34 cDNAs as probes. The pHIG-34 cDNA probe exhibited exactly the same hybridization pattern as did the pHIG-1 cDNA probe, whereas similar but not identical results were obtained with the pHIG-15 cDNA probe (Fig. 1B) and all the hybridization bands shown in Fig. 1A and B were detected with the pHIG-2 cDNA probe (data not shown). These hybridization data are summarized in Fig. 2A and indicate that these four cDNA clones contain the DNA sequences complementary to a common repeat unit or to a common multicopy extra-chromosomal unit, such as mitochondrial DNA. The structural features of human mitochondrial DNA (11) are consistent with the hybridization pattern observed (compare Fig. 2A and B).

Restriction Mapping of the cDNA Clones. To further examine the relationship between these cDNA clones and the mitochondrial DNA, we constructed detailed cleavage maps of the pHIG-1, pHIG-2, pHIG-15 and pHIG-34 DNAs. Fig. 3 summarizes the restriction maps of these four cDNA clones (Fig. 3A) and the

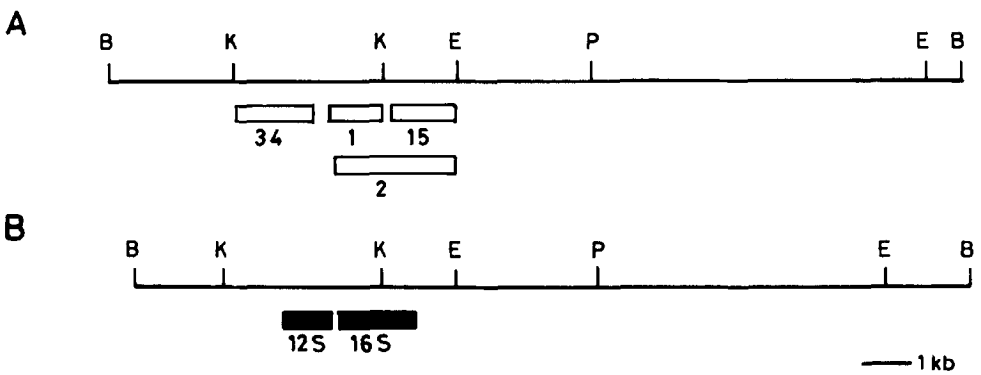

Fig. 2. A restriction map of the genomic DNA (A) and a relevant restriction map of human mitochondrial DNA (B) (11). The map of the genomic DNA was constructed from the Southern blot analyses. Four open boxes indicate the DNA regions hybridizing with pHIG-34, pHIG-1, pHIG-15, and pHIG-2 cDNAs, and two solid boxes represent 12S and 16S ribosomal RNA genes, respectively. The other symbols are the same as in Fig. 1.

published restriction map of the mitochondrial 12S and 16S ribosomal RNA gene region (Fig. 3B) (11). Among the 25 restriction enzyme cleavage-sites present on these four cDNA clones, we can assign 19 to the corresponding sites on the mitochondrial ribosomal RNA gene region. The results shown in Fig. 3 indicate that three independently isolated clones, i.e., pHIG-1, pHIG-15, and pHIG-34,

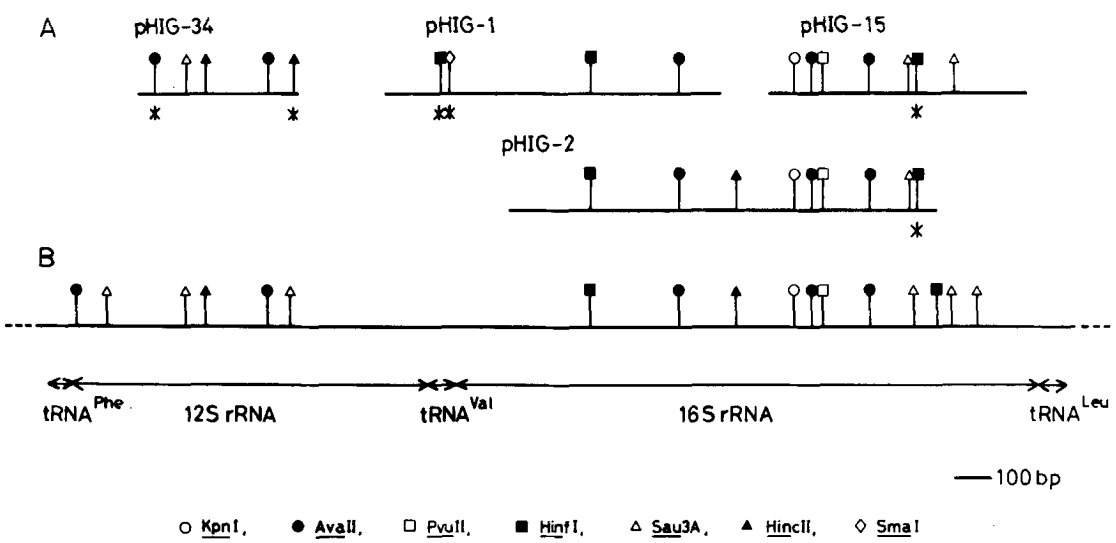

Fig. 3. Restriction maps of four cDNA clones and human mitochondrial DNA.

A, cDNA clones. pHIG-34, pHIG-1, pHIG-15, and pHIG-2 were described in Table 1.

B, a map of the human mitochondrial ribosomal RNA gene region (11). The restriction sites are indicated by symbols. The symbols with an asterisk at the bottom indicate the restriction sites that are not found on the corresponding sites of the mitochondrial ribosomal RNA gene region.

which do not hybridize with each other, represent transcripts of different parts of mitochondrial ribosomal RNA genes, and are consistent with the finding that the cDNA insert isolated from pHIG-2, one of the class III clones, can hybridize with both pHIG-1 and pHIG-15 (Table 1). These results demonstrate that our cDNA clones closely resemble human mitochondrial ribosomal RNA genes.

Characterization and sequencing of the cDNA clones are underway to determine whether they are derived from mitochondrial DNA transcripts or whether they represent transcripts of mitochondrial DNA-like sequences present in nuclear DNA.

In addition, these results indicate that approximately 80% of the dense positive colonies could have been eliminated by screening with mitochondrial DNA or by including human mitochondrial DNA or RNA in excess during the initial screening procedures (11). The efficiency of such primary screening procedures can thus be increased and the work involved in identifying specific clones by hybridization selection or hybrid-arrested translation reduced accordingly. As applied to our original isolation of human leukocyte and fibroblast interferon cDNA clones (1), it would have been necessary to screen only 73 colonies by hybridization selection if a mitochondrial DNA sequence was included in the hybrid-ization medium to exclude these clones. Identification of the remainder of the dense positive colonies is still underway.

ACKNOWLEDGMENTS: We thank Drs. M. Nakayama and M. Maeyama, Dept. of Obstetrics and Gynecology, Kumamoto University, for provision of the human placenta and M. Ohara for reading the manuscript. This work was supported in part by a scientific grant from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Maeda, S., McCandliss, R., Gross, M., Sloma, A., Familletti, P.C., Tabor, J.M., Evinger, M., Levy, W.P., and Pestka, S. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7010-7013.
2. Blin, N., and Stafford, D.W. (1976) *Nucleic Acids Res. 3*, 2303-2308.
3. Mukai, T., Ohkubo, H., Shimada, K., and Takagi, Y. (1978) *J. Bacteriol. 135*, 171-177.
4. Southern, E.M. (1975) *J. Mol. Biol. 98*, 503-517.
5. Smith, G.E., and Summers, M.D. (1980) *Anal. Biochem. 109*, 123-129.
6. Wahl, G.M., Stern, M., and Stark, G.R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3683-3687.
7. Thomas, P.S. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201-5205.

8. Rigby, P.W.J., Dieckmann, M., Rhodes, C., and Berg, P. (1977) *J. Mol. Biol. 113*, 237-251.
9. Griffin-Shea, R., Thireos, G., Kafatos, F.C., Petri, W.H., and Villa-Komaroff, L. (1980) *Cell 19*, 915-922.
10. Grunstein, M., and Hogness, D.S. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3961-3965.
11. Anderson, S., Bankier, A.T., Barrell, B.G., de Bruijn, M.H.L., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R., and Young, I.G. (1981) *Nature 290*, 457-465.