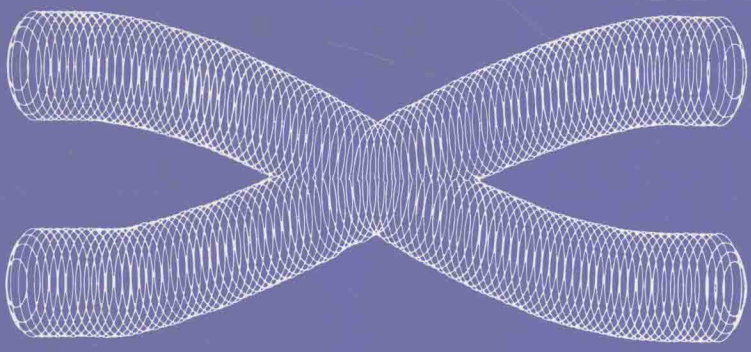
RESEARCH PERSPECTIVES IN CYTOGENETICS



Edited by
ROBERT S. SPARKES and
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Preface

National awareness of the need for a concentrated attack on the problem of mental retardation led, in 1963, to legislation authorizing construction grants for Mental Retardation Research Centers in which biological, medical, behavioral, and social science research and research training relating to mental retardation could be conducted. Implementation of this legislation by the National Institute of Child Health and Human Development (NICHD) gave recognition to the unique demands posed by the complex problem of mental retardation—a product of diverse etiologies, both biological and behavioral, extending over the life span from conception to maturity, and requiring ameliorations as well as preventive research efforts.

The NICHD subsequently funded twelve mental retardation research centers. One of these centers was established at the Center for the Health Sciences, University of California, Los Angeles (UCLA). In 1970, the UCLA Mental Retardation Research Center sponsored jointly with the NICHD's Mental Retardation and Developmental Disabilities Branch a conference entitled Perspectives in Cytogenetics: The Next Decade. The purpose of this conference was to evaluate the field and anticipate potential progress in the next ten years. That meeting consolidated burgeoning cytogenetic knowledge including, among others, new developments in prenatal diagnosis, chromosome anomalies after oral contraceptive use, cytogenetic registries, the application of computer technology in cytogenetics, and fluorescent banding of human chromosomes. Recommendations growing out of the meeting have served to guide NICHD programming in cytogenetics to the present.

In keeping with its identification of research in cytogenetics as one of its priority programs, the NICHD Mental Retardation and Developmental Disabilities Branch sponsored jointly with the UCLA Mental Retardation Research Center a follow-up meeting, given the title Research Perspectives in Cytogenetics, on August 27–28, 1981, at the UCLA Center for the Health Sciences. This meeting assessed recent progress in cytogenetics, especially human cytogenetics. The variety of topics discussed emphasized the important contributions which are influencing our understanding of the structure and function of the human chromosome.

The product of this meeting is presented in this publication, which is the 12th volume in the Mental Retardation Research Centers Series. Through the series, the Mental Retardation Research Centers and the NICHD express their joint responsibility for the dissemination of new research knowledge and for drawing attention to emerging research needs and opportunities. Collectively, the series has addressed a range of research developments from the medical, biomedical, behavioral, social, and communications sciences. We hope that the information contained in this volume will contribute to still further progress in cytogenetic research over the next decade.

We wish to thank the many participants for contributing to the success of the meeting and to offer special thanks to David Comings, Park Gerald, and John Hamerton, who served on the planning committee with us.

Robert S. Sparkes, M.D.

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Contents

DNA Restriction Fragment Length Polymorphisms in Human Leukemic Leukocytes

Grady F. Saunders, Bruno Calabretta, Donald L. Robberson, Hugo A. Barrera-Saldana, and Thymios P. Lambrou

A family of highly repetitive DNA sequences, the Alu repeat family, is the predominant repetitive sequence in humans (1, 2). By renaturation kinetics (3) and by screening of a genomic library (4), it has been shown that the human genome contains at least 400,000 copies of the 300-bp Alu repeat sequence. There are many interesting features that suggest that members of the Alu repeat family may serve cellular functions. These features include the widely dispersed arrangement of Alu repeats, the conservation of a 40-bp segment of the Alu repeat in mammalian evolution, the considerable homology of a region 14 nucleotides in length with a sequence near the replication origin in several DNA viruses, and the observation that the Alu repeat is represented in transcripts generated by both DNA-dependent RNA polymerases II and III (5, 6). Thus, a role for Alu repeat sequences in the initiation of DNA replication and nuclear RNA processing has been postulated (5, 6).

The most striking feature of the Alu repeat family is its large numerical representation in the human genome, which suggests that Alu repeat sequences might be involved in genetic rearrangements. Therefore, if we consider the human genome to be a dynamic structure, we should be able to detect involvement of Alu repeat sequences in DNA rearrangements. Although most members of the Alu family are scattered throughout the human genome, some of them may be clustered in certain genomic regions. Finding such a region would provide a good opportunity to test the hypothesis that Alu repeat clusters may be involved in genetic rearrangements. Portions of this work have been reported in a previous communication. (7).

This investigation was supported by grants from the National Institutes of Health (GM 23965, GM 27774, CA 20124, CA 16672, and CA 16527) and the Robert A. Welch Foundation (G-267 and G-841). H.A.B.S. was supported by a scholarship from the Consejo Nacional de Ciencia y Tecnología (CONACYT) of the government of Mexico.

EXPERIMENTAL PROCEDURES

Recombinant clone λH15 was isolated from a human genomic library (8) as a clone hybridizing to total nick-translated repetitive sequences, grown as previously described (4), and the human insert was characterized with respect to the distribution of repetitive sequences. The orientation of the human insert with respect to the left and right arms of the vector (9) was facilitated by knowing the restriction map for Charon 4A. The localization of Alu repeat sequences was initially derived for the restriction map of λH15, and confirmed for the restriction map of several segments of λH15 (subcloned in to pBR322 or pACYC184) using Southern blot hybridization (10) with nick-translated Alu repeat inserts from either the clone BLUR 8 or BLUR 2 (2) as previously described (6).

Nuclear DNA was prepared from human cells and tissues as previously described (11). Radioactive labeling of cloned DNA, restriction enzyme digestion of DNA, and Southern transfer and blot hybridization were carried out as previously described (7). Hybridization was performed in dextran sulfate as previously described (12).

RESULTS

Alu Repeat Sequences Are Clustered in Some Regions of the Human Genome

We searched a human genomic DNA library (8) for clones containing several Alu repeat sequences. Previously, we showed that more than 95% of this library contains members of the Alu repeat family (4), and in several clones two or more copies are present (G. Saunders et al., unpublished observation). Clone λH15, randomly selected as strongly hybridizing to repetitive sequences, was examined in detail. An initial digestion of this clone with several restriction enzymes followed by Southern blot hybridization with the cloned Alu repeat sequence probes BLUR 2 and BLUR 8 (2) gave several hybridizing bands, indicating that many Alu repeat family members were present in this 15-kb DNA insert. Double digestion with restriction endonucleases Eco RI plus Bam HI and Eco RI plus Pst I showed 7 and 12 bands, respectively, hybridizing to these cloned members of the Alu repeat family.

The restriction enzyme cleavage map of λH15, along with the approximate locations of the Alu repeat family members, is shown in Figure 1. At least 10 Alu repeat sequences are clustered in this clone; this is a minimal estimate due to the high degree of divergence among different members of the Alu repeat family (2). Two observations give credence to this assertion. First, we identified an Alu repeat sequence hybridizing very strongly to BLUR 2 (dark box in Figure 1) and very weakly to BLUR 8. The sequence divergence between BLUR 8 and BLUR 2 is 24%, which precludes cross-hybridization under the conditions used. This observation suggests that some members of the Alu repeat family have diverged considerably in the Alu repeat cluster of λH15. Second, in R-loop hybridization between poly (A$^+$)RNA, derived from a human lymphoblastoid line, and subclone pλH15C (Figure 1), two additional Alu-like sequences have been detected and positioned with respect to the Alu sequence distribution derived by Southern blot analysis (6). We may, therefore, consider the segment of

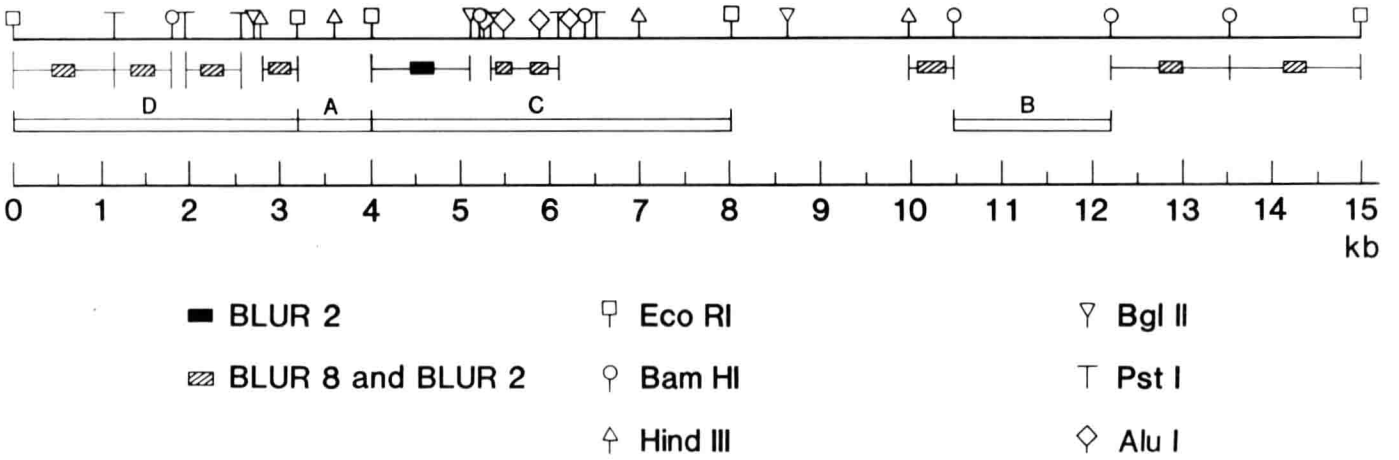

Figure 1. Anatomy of recombinant clone λH15. The 15-kb human DNA insert of recombinant clone λH15 with cleavage sites for several restriction enzymes is shown. The positions of Alu repeat sequences are indicated. Subclones of λH15 were prepared containing the Eco RI fragments A, B, C, and D.

λH15 between the first and the fourth Eco RI sites (Figure 1) as a cluster of Alu repeat sequences.

Heteroduplexes formed between subclone pλH15C and a second subclone pλH15D, both of which are described in Figure 1, showed extensive homology except for two regions in which a small loop is detected (Figure 2). The loop that is 0.26 ± 0.02 of the distance from the terminus could represent a deletion or addition of a sequence

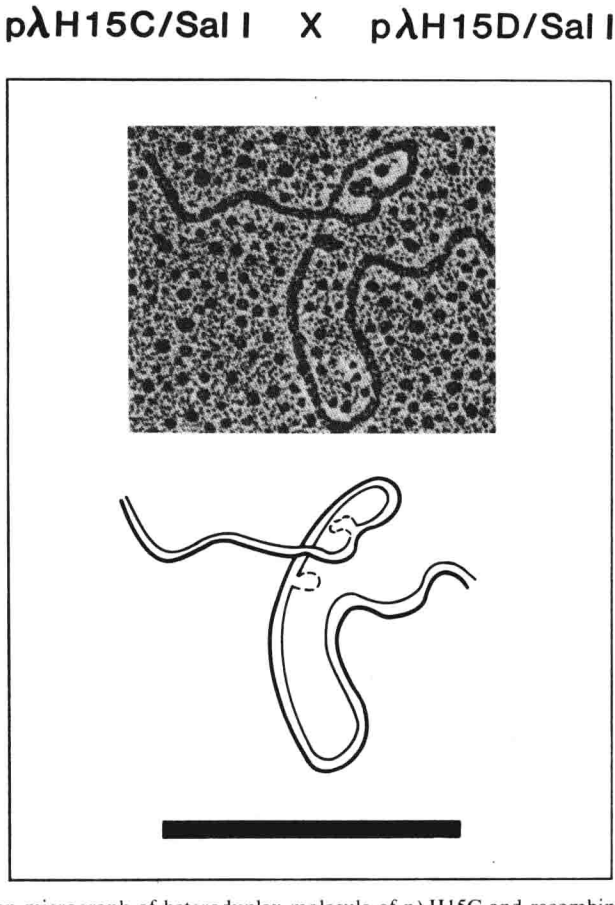

Figure 2. Electron micrograph of heteroduplex molecule of pλH15C and recombinant DNAs. Eco RI fragments C and D from λH15 (Figure 1) were subcloned in plasmid vector pACYC 184. In the heteroduplex depicted, sequences corresponding to the inserted Eco RI fragments begin 2 kb from each terminus and are 4 kb (pλH15C) and 3.2 kb (pλH15D) in length. The heteroduplexes appeared to be fully base-paired except for one or two regions where a single-strand loop of DNA occurred, as indicated by dashed lines in the drawing below the electron micrograph. The most frequently occurring position of this loop was at 0.26 ± 0.02 of the heteroduplex length from one terminus (loop at left). The average length of DNA in this loop was estimated to be 300–600 bases. Less frequently, a loop of single-stranded DNA appeared at 0.41 ± 0.02 (loop at right) or at 0.32 ± 0.02 (not shown). The second, less frequent loop also had a length of 300–600 bases. Although most heteroduplexes contained only one detectable loop, several examples containing two loops at the indicated positions were found. Heteroduplexes were identified by their duplex length relative to PM2 DNA, which has a length of 10,260 bp. Bar inset represents 0.5 μm.

of 300–600 bp from pλH15C or pλH15D, respectively, that occurs very near the junction (0.25 of the distance from the Sal I terminus) with the linearized vector DNA. This loop is not always discernible because of its small size. The position and length of this loop are in good agreement with the position and difference in length (600 bp) of the shortened Eco RI–Hind III fragment of pλH15D. This suggests that a deletion in λH15D or an addition in λH15C of approximately 500 nucleotides has occurred in this segment. A second loop is sometimes detected at either 0.41 ± 0.02 or 0.32 ± 0.02 of the distance from the same Sal I terminus, as deduced from a few examples containing two loops, as shown in Figure 2. We believe that the second loop corresponds to the addition of a sequence in pλH15D derived by duplication of a sequence present at the same site in both pλH15D and pλH15C. The alternative positions of the loop, corresponding to positions at 2.0 and 2.6 kb, respectively, on the map in Figure 1, therefore represent alternative nonhybridized sites of this sequence as would occur for a tandem duplication in one of the two DNAs in the heteroduplex. It should be emphasized that the heteroduplexes would not reveal smaller regions of inhomology (less than approximately 100 bp) that may exist for these two subcloned DNA fragments.

Because of the observed instability of cloned repetitive or duplicated sequences in REC A *Escherichia coli* hosts (13, 14), some caution is necessary in proposing that additions or deletions of sequences are derived from the genomic DNAs. The possibility that these deletions or additions are present in genomic DNA and not derived from propagation in *E. coli* is strongly indicated from the results obtained using pλH15A (Figure 1) as a probe to survey the sequence arrangement in restriction enzyme fragments that encompass this region of the human genome. The same-size DNA fragments containing H15 sequences were present in Bam HI, Hind III, Pst I, Eco RI, and Bgl II digests of both placental DNA and λH15 (data not shown). This result indicates that the segments comprising the loop structures in heteroduplexes formed between DNA of clones pλH15C and pλH15D correspond to either deletions or additions in genomic DNA.

DNA Restriction Fragment Length Polymorphisms (RFLP) and Differential Copy Number of Sequences Hybridizing with pλH15A

These findings suggested that rearrangements had occurred in this region of the genome and that Alu repeat clustering may have played a role in these events. To investigate this possibility we took advantage of the observation that in λH15 there are DNA segments of single copy or very low repetition frequency flanked on both sides by Alu repeats. Subclones of two of these regions (A and B in Figure 1) were constructed for use as hybridization probes to detect RFLPs in the genomic region in which the human insert of λH15 is localized. If DNA rearrangements occur in this region at a relatively high frequency, they should be detectable by RFLP analyses. We therefore examined the DNA from tissues of healthy individuals, as well as patients with neoplastic hematological diseases, by cleavage with restriction enzymes and blot hybridization experiments. Substantial polymorphism was observed with DNA from tissues of healthy individuals (Figure 2). Digestion of several of the DNA samples with other restriction enzymes also revealed patterns of polymorphism. In addition to the detection of RFLP, a marked difference in the intensity of one hybridizing band exists among

DNAs derived from leukocytes of a normal donor (Figure 3, left side lane C) and DNA derived from leukemic leukocytes (lanes D through I). These results indicate both variation in the number of hybridizing sequences (copy number) and multiple forms revealed by RFLP of the sequence in human DNA that hybridizes to pλH15A.

To determine and compare the number of sequences hybridizing to pλH15A in DNAs derived from leukocytes of a patient with acute lymphocytic leukemia and a normal individual, a gene copy number reconstitution experiment was performed. The method used has sufficient sensitivity and reproducibility to give reliable estimates of gene copy numbers (15, 16). Titration experiments (Figure 4) show that more than 50 copies of sequences hybridizing to pλH15A are present in the leukocytes of this normal individual, whereas fewer than five copies are present in leukocytes of the leukemic patient. These results indicate that significant differences may exist in the number of

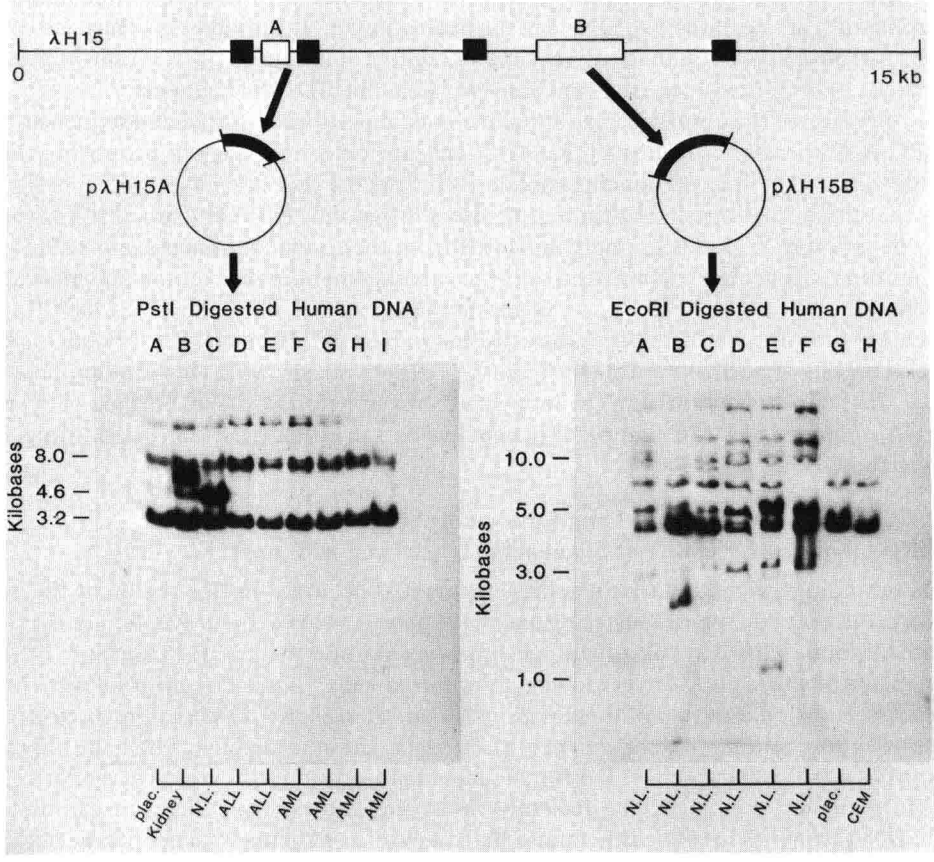

Figure 3. DNA rearrangement in normal and leukemic individuals. The dark boxes on the human inset of λH15 represent Alu repeat sequences flanking the DNA fragment present in subclone pλH15A. Ten micrograms of human DNA was digested with Pst I (left side) or Eco RI (right side), electrophoresed through 1% agarose gels, and transferred to nitrocellulose. The resulting blots were hybridized with labeled pλH15A and pλH15B, respectively, and the hybrids were exposed to x-ray film. AML, acute myelogenous leukemia; N.L., normal leukocytes. Reprinted with permission from Calabretta et al. (*Nature* 295:219–225, 1982).