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Triplet repeat sequences in human DNA can be detected by hybridization to a synthetic (5′-CGG-3′)$_{17}$ oligodeoxyribonucleotide

Annett Behn-Krappa, Jan Mollenhauer, Walter Doerfler*

Institute for Genetics, University of Cologne, Weyertal 121, D-5000 Cologne, Germany

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The seemingly autonomous amplification of naturally occurring triplet repeat sequences in the human genome has been implicated in the causation of human genetic disease, such as the fragile X (Martin–Bell) syndrome, myotonic dystrophy (Curshmann–Steinert), spinal and bulbar muscular atrophy (Kennedy's disease) and Huntington's disease. The molecular mechanisms underlying these triplet amplifications are still unknown. We demonstrate here that a synthetic (CGG)$_{17}$ oligodeoxyribonucleotide can be utilized as hybridization probe to visualize some of the triplet repeats in the human genome. This technique may help in studies aimed at the elucidation of the amplification mechanism.

Triplet repeat amplification; (CGG)$_x$ repeat; Human DNA

1. INTRODUCTION

Naturally occurring triplet repeat sequences in the human genome can become amplified severalfold by mechanisms that are presently not understood. Several well known human genetic diseases, like the fragile X (Martin–Bell) syndrome, myotonic dystrophy (Curschmann–Steinert), the spinal and bulbar muscular atrophy (Kennedy's disease), and Huntington's disease have been causally related to the amplification of the triplet repeats CGG, GCT, and CAG, respectively (for reviews, see [1–3]). Since triplet repeats have been observed within or close to a number of human genes by a gene bank search, it is conceivable that triplet amplifications may be involved in the causation of other genetic diseases as well [1,4].

A simplified assay for triplet repeat analyses might facilitate the initiation of projects aiming at the elucidation of the mechanism of triplet amplifications in the human genome.

2. MATERIALS AND METHODS

The synthetic (CGG)$_{17}$ oligodeoxyribonucleotide has been used as hybridization probe in the visualization of these repeats in human DNA by alkaline downward Southern [5] blotting [6]. The 51-mer oligodeoxyribonucleotide (CGG)$_{17}$ was synthesized in an Applied Biosystems 381A DNA Synthesizer and labeled at the 5′ end by using [γ-^{32}P]ATP (6000 Ci/mmol) and polynucleotide kinase [7].

Cellular DNAs from primary human cells or from cells growing in culture were extracted by the standard SDS-proteinase K-phenol/chloroform procedure [8] as described earlier [9]. Human lymphocytes were stimulated with phytohemagglutinin and interleukin-2. The nucleic acid preparations were treated with RNase during the incubation with the different restriction endonucleases. Control DNA preparations, as human adenovirus type 12 (Ad12) DNA [10], pBR322 plasmid DNA [11], pBluescript KS DNA (Stratagene), DNA from three cloned fragments (HRI, Hind-F, pE38) of *Autographa californica* nuclear polyhedrosis virus DNA [12], the cloned *Pst*I-A fragment of Ad12 DNA in the pBluescript KS vector [13], the cloned E2A late promoter of Ad2 DNA [14], or the human c-myc promoter [15] were prepared as described in the cited references.

DNA preparations were cleaved with restriction endonucleases as described in the figure legends or figures, the DNA fragments were separated by electrophoresis on 1.2 to 1.5% agarose gels, and transferred to Hybond N$^+$ or Quiabrene$^+$ membranes in 0.4 M NaOH for 4 h by the downward blotting procedure [6]. The DNA was hybridized overnight to the ^{32}P-labeled probe in 2 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M Na-citrate), 1% SDS, 0.5% fat-free milk powder, 0.5 mg salmon sperm DNA per ml, 10% dextran sulfate in a rotating tube at 61°C. Subsequently, the membranes were washed for 15 min each at 61°C in rotating tubes in (i) 2 × SSC, 1% SDS; (ii) 1 × SSC, 1% SDS; (iii) 0.75 × SSC, 0.75% SDS, and (iv) 0.5 × SSC, 0.5% SDS. The filters were autoradiographed at −70°C on Kodak XAR films.

In some experiments, the ^{32}P-labeled (CGG)$_{17}$ probe was subsequently removed by boiling for 30 min in 0.1 × SSC, 0.1% SDS, and the DNA was rehybridized to a ^{32}P-labeled [16] c-myc or pBR322 probe.

3. RESULTS AND DISCUSSION

DNA from the lymphocytes of several different individuals, from human sperm, placenta or from established cell lines (HeLa, DEV, Jurkat) was cleaved with restriction endonucleases as indicated, the fragments were electrophoretically separated and hybridized to the triplet repeat probe. Representative results shown in Fig. 1 documented the presence of distinct bands whose sizes, of course, depended on the restriction enzymes used. Most of the DNA fragments ranged in size between 0.5 and 5 kilobase pairs (kbp). Hybridization patterns were either simple, e.g. upon *Sac*I cleavage of

*Corresponding author. Fax: (49) (221) 470 5163.

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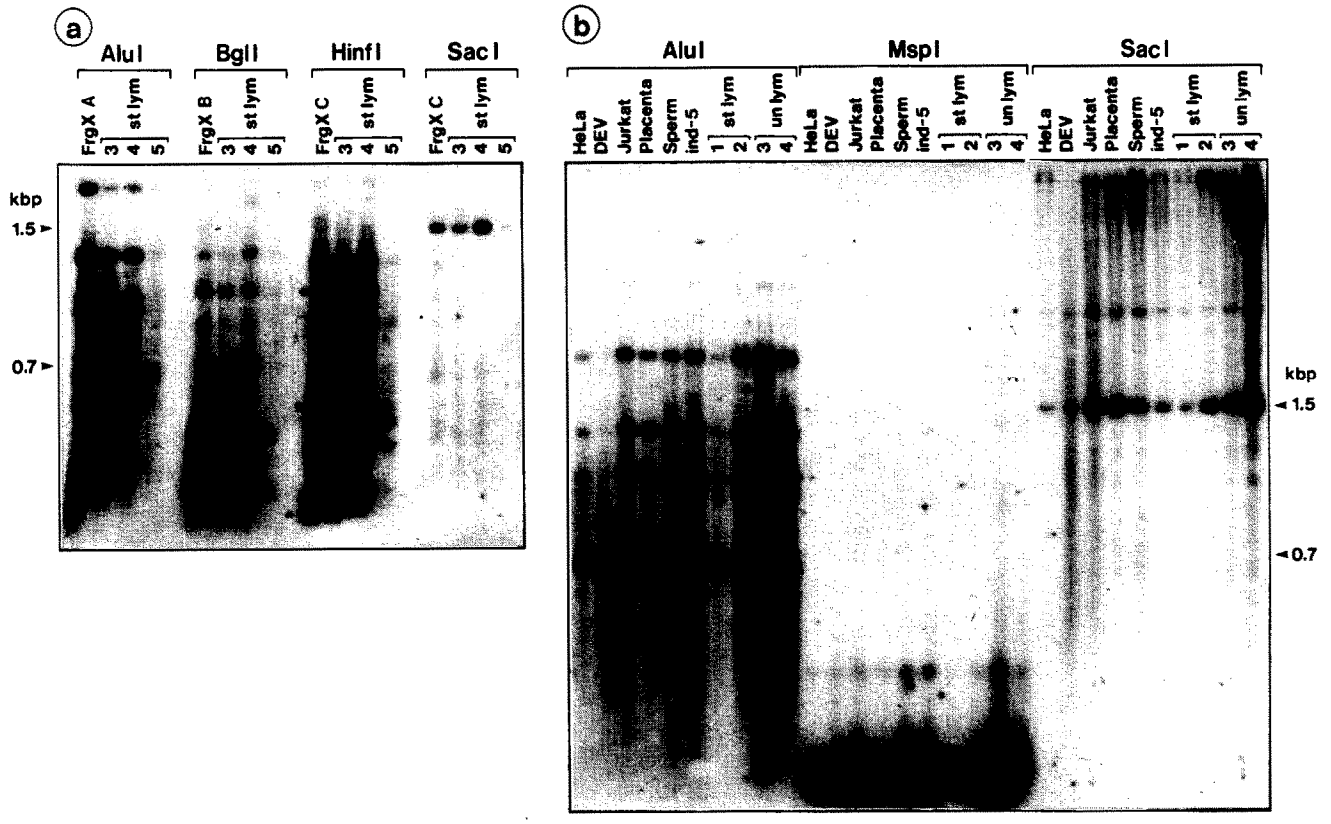

Fig. 1. Autoradiograms of two Southern blots demonstrating the hybridization of the ^{32}P-labeled (5′-CGG-3′)$_{17}$ oligodeoxyribonucleotide probe to specific segments in several different human DNA preparations from sources as indicated (see below for abbreviations). DNA fragments were electrophoretically separated on a 1.2% agarose gel. Experimental details were described in the text. The autoradiograms were exposed at −70°C for 3 (a) or 10 (b) days. *Abbreviations:* FrgX, Lymphocyte DNA from three different fragile X patients (A–C); st lym, DNA from stimulated lymphocytes; un lym, DNA from unstimulated lymphocytes; ind-5, DNA from total blood of one individual; 1–5, DNA samples from different individuals. Size scales were presented on the right and left margins.

the genomic DNA or complex, e.g. after cleavage with *Hin*fI. Most cleavage patterns of DNAs from different individuals were very similar, including those of three different fragile X patients. Deviations in the patterns possibly indicating polymorphisms were occasionally observed (asterisks in Fig. 1). Hybridization patterns of the (CGG)$_{17}$ oligodeoxyribonucleotide with cellular DNA from primary cells and from established cell lines were very similar.

It is concluded that the synthetic oligodeoxyribonucleotide (CGG)$_{17}$ can be used as hybridization probe to cellular DNA sequences abundant in this triplet repeat sequence. We have initiated experiments to clone and further characterize the DNA sequences containing these repeats.

The autoradiogram in Fig. 2a demonstrated the results of control experiments. The ^{32}P-labeled (CGG)$_{17}$ oligodeoxyribonucleotide as hybridization probe visualized distinct segments in the *Hin*fI-cleaved DNA from interleukin-2 and phytohemagglutinin-stimulated human lymphocytes. In contrast, the DNA from plasmids pBR322, pBluescript KS, from particular fragments of the DNA from *Autographa californica* nuclear polyhedrosis virus (HR1, Hind-F, PE38; [12]), from Ad2 DNA (E2A late promoter; [14]), from Ad12 DNA (data not shown) and from the *Pst*I-A fragment of Ad12 DNA [13] or from part of the human c-myc promoter DNA [15], containing a CpG island, did not hybridize to the 51-mer repeat probe. The DNA in the 'empty' lanes of Fig. 2a hybridized to ^{32}P-labeled pBR322 DNA (Fig. 2b) attesting to the fact that the 'empty' lanes did contain DNA. The results of these control experiments documented the specificity of the (CGG)$_{17}$ hybridization probe.

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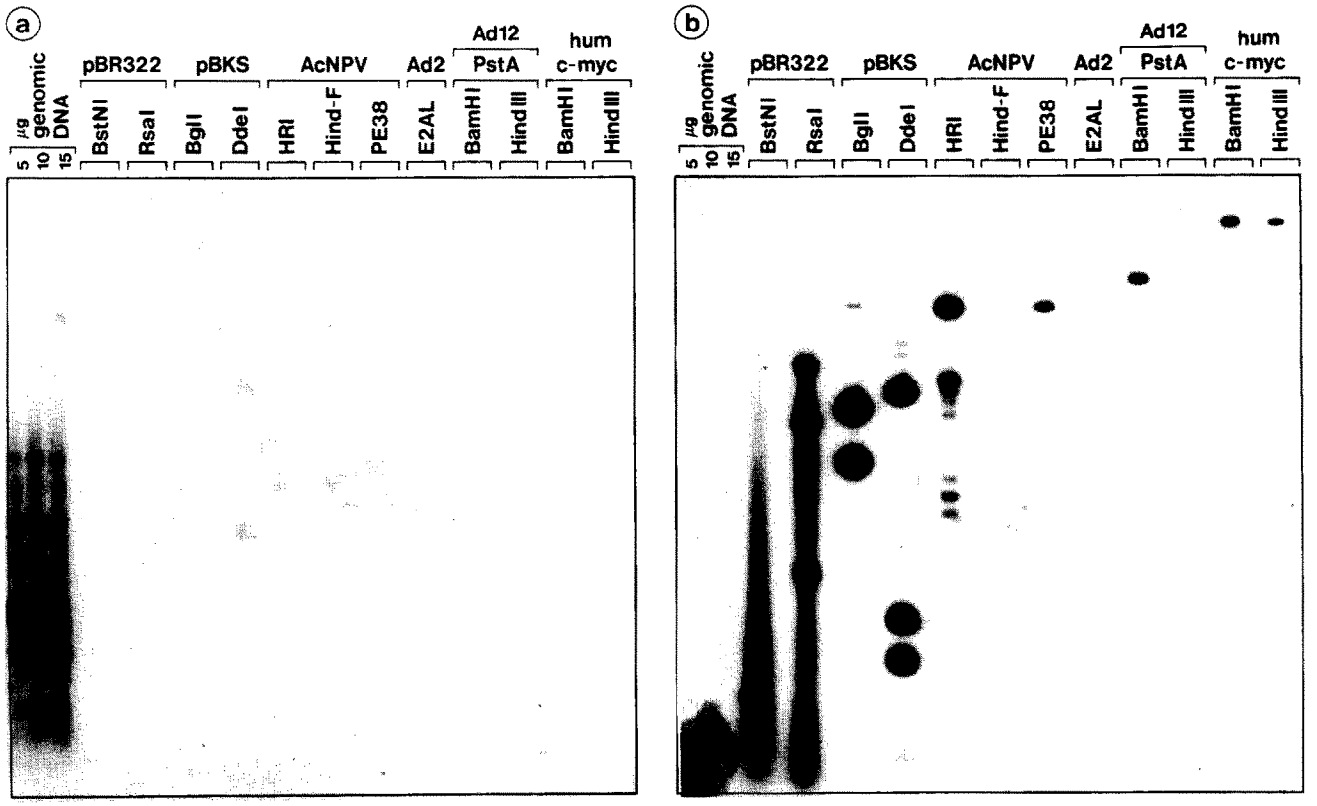

Fig. 2. Control autoradiograms demonstrating the specificity of the hybridization reaction with the ^{32}P-labeled $(5'\text{-CGG-}3')_{17}$ oligodeoxyribonucleotide probe. The amounts of DNA (alternatingly left to right, 1 ng–1 pg) and its derivations were indicated for each slot. DNA fragments were separated by electrophoresis on a 1.5% agarose gel. Experimental details were described in the text. The autoradiograms were exposed at −70°C for one day each. Hybridization probes were the ^{32}P-labeled $(5'\text{-CGG-}3')_{17}$ oligomer (a), or pBR322 DNA (b). *Abbreviations:* pBKS, DNA from plasmid Bluescript KS; AcNPV, DNA from *Autographa californica* nuclear polyhedrosis virus; hum c-myc, DNA from the human c-myc promoter.

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