Vol. 137, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1986 Pages 474-479

AFFINITY ISOLATION OF TRANSCRIPTIONALLY ACTIVE DNA

Barry Roseman, John Lough, Everin Houkom and Tim Herman[1]

Department of Anatomy and Cellular Biology and [1]Department of Biochemistry
Medical College of Wisconsin, Milwaukee, WI 53226

Received April 16, 1986

Chicken erythrocyte nuclei were nick translated with the chemically cleavable biotinylated nucleotide, Bio-12-SS-dUTP. DNA was purified, digested with restriction endonucleases, and applied to an avidin-agarose affinity column. Seventy percent of the nick translated DNA bound to the column. This DNA was recovered from the column by chemical cleavage of the linker arm joining biotin to the DNA. Dot hybridization analysis of this DNA revealed a significant enrichment of the alpha-D-globin gene. This result suggests an approach to isolate transcriptionally active genes.

Transcriptionally active genes are known to be more sensitive to nuclease digestion than nontranscribed DNA sequences (1). Levitt et. al.(2) demonstrated that when isolated chick oviduct nuclei are nick translated in a way analogous to that used to radiolabel purified DNA, over 80% of the labeled DNA sequences represent genes that were transcriptionally active in that tissue. This approach has been subsequently used to demonstrate that active genes in isolated metaphase chromosomes retain an increased sensitivity to DNase I (3), to distinguish between transcriptionally active and inactive X chromosomes (4,5) and to localize transcriptionally active domains of chromatin in intact nuclei (6).

The chemically cleavable biotinylated nucleotide, Bio-12-SS-dUTP, can be used to affinity label purified DNA by nick translation (7). The biotinylated DNA can be specifically bound to an avidin-agarose affinity column and subsequently recovered by chemical cleavage of the linker arm joining the

Abbreviations: Bio-12-SS-dUTP, a biotinylated nucleotide analog containing a disulfide bond in the 12-atom linker arm joining biotin to the 5-C of the pyrimidine base uracil (7); DTT, dithiothreitol.

0006-291X/86 $1.50

biotin to the DNA (8). In this paper we demonstrate that the alpha^{D}-globin gene in chicken erythrocyte nuclei can be similarly affinity labeled and chromatographed to yield a fraction of DNA that is enriched in the alpha^{D}-globin gene.

METHODS

Nick Translation of Nuclei. Nuclei were isolated from adult chicken erythrocytes (9) and suspended in buffer A (10mM Tris, pH 7.5, 10mM NaCl and 3mM $MgCl_2$) at a concentration of 1.5 X 10^8 nuclei per ml. Five hundred microliter aliquots were incubated with DNase I (0 to 0.2 µg/ml) for 15 min at 25 °C. The nicked nuclei were washed twice with buffer A to remove the DNase I and resuspended in 500 µl of the same buffer. Each polmerization reaction contained 75 X 10^6 nuclei in 500 µl buffer A containing 20 µM dATP, dGTP and dCTP, 5 µM ^{3}H-dTTP (18µCi/ml), 15 µM Bio-12-SS-dUTP and 25 U of E. coli DNA polymerase I. Reactions were incubated for 20 min at 15 °C. Aliquots were removed during the reaction to measure the incorporation of ^{3}H-dTTP into TCA-precipitable DNA. Following nick translation, the nuclei were washed twice with Buffer A to remove unincorporated nucleotides. DNA was purified from the nuclei, incubated with RNase A and digested with Eco RI and Hind III using standard procedures. Bio-12-SS-dUTP was synthesized and purified as reported previously (10).

Avidin-Agarose Affinity Chromatography. Fifty microgram aliquots of the DNA purified from nick translated nuclei were incubated for 1 hr at room temperature with 500 µl Avidin-D Agarose (Vector Laboratories) in TE buffer (10mM Tris, pH 7.5, 1mM EDTA) supplemented with 50 mM NaCl. The avidin agarose resin was gently pelleted by centrifugation and the supernatant was removed. The resin was washed once with 500µl TE buffer containing 50mM NaCl, three times with TE buffer containing 200mM NaCl, and two times with TE buffer containing 200mM NaCl and 50mM dithiothreitol (DTT). The pH of the DTT-containing buffer was increased to 8.2 to increase the rate of disulfide bond cleavage. The affinity resin was incubated at room temperature for 15 min in the DTT-containing buffer prior to centrifugation of the resin and removal of the supernatant. Supernatants from the first five washes (no DTT) contained the "unbound" DNA and were pooled. Supernatants from the two DTT washes contained the affinity-labeled DNA that was bound to the avidin-agarose and released following DTT cleavage; these fractions were pooled and referred to as the "bound" DNA.

Dot hybridization analysis of fractionated DNA. The concentration of DNA in the unbound and bound fractions was determined by Hoechst dye assay (11). Serial two-fold dilutions of the DNA were applied to nitrocellulose paper (BA85, Schleicher and Schuell) and prepared for dot hybridization (12). Plasmid DNA (pBR322) containing a 1.4 kb genomic alpha^{D}-globin insert (14) was nick translated with ^{32}P-dCTP to a specific radioactivity of 2.5 X 10^8 dpm per µg(13). Hybridization and subsequent detection of the radiolabeled probe were performed by standard procedures (12).

RESULTS

Nuclei were isolated from chicken erythrocytes and nicked with increasing concentrations of DNase I prior to incubation with E. coli DNA polymerase I and a nucleotide mixture containing Bio-12-SS-dUTP. As shown in Figure 1, the incorporation of ^{3}H-dTTP into DNA was dependent on the concentration of DNase

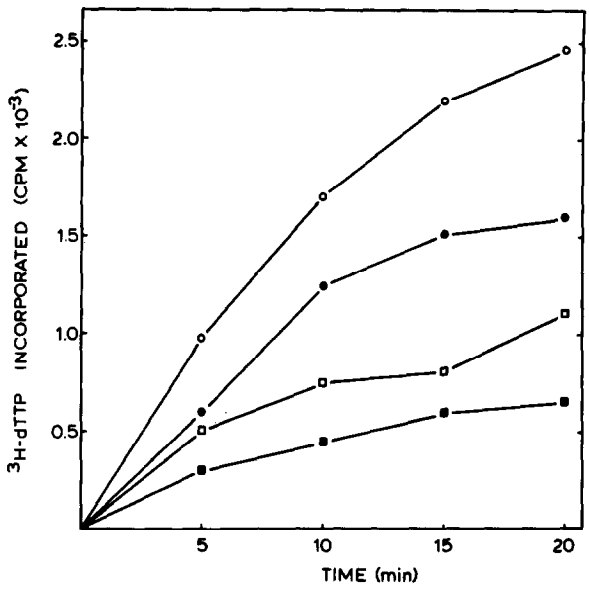

Figure 1. Nick translation of chicken erythrocyte nuclei. Reactions were performed as described using no DNase I (■), 0.05 µg DNase I per ml (□), 0.1 µg DNase I per ml (●) or 0.2 µg DNAse I per ml (○).

I. Agarose gel electrophoresis of the DNA following DNase I digestion revealed a reduction in the average size of the DNA only at DNase I concentrations greater than 0.5 µg per ml (data not shown).

DNA was purified from nick translated nuclei, fragmented by digestion with the restriction endonucleases Eco RI and Hind III, and chromatographed on avidin-agarose. A typical chromatographic profile of DNA isolated from nuclei nick translated using 0.2 µg/ml DNase I is shown in Figure 2 . The DNA mass present in each wash was measured by fluorometric assay (open bars) and expressed as a percentage of the total nuclear DNA. Ninety-four per cent of the total DNA was recovered in the first five washes, constituting the "unbound" fraction. Of this, 22% was recovered in washes containing 50mM NaCl, whereas the remaining 72% was released when the NaCl concentration was increased to 200mM. This requirement for 200mM NaCl to release nonspecifically bound DNA has been noted previously (8) and is due to the basic nature of avidin (pI=10.5). The remaining 6% of the total DNA which was released in the two washes containing 50mM DTT constituted the "bound" DNA fraction. Although comprising only 6% of the total nuclear DNA, the bound

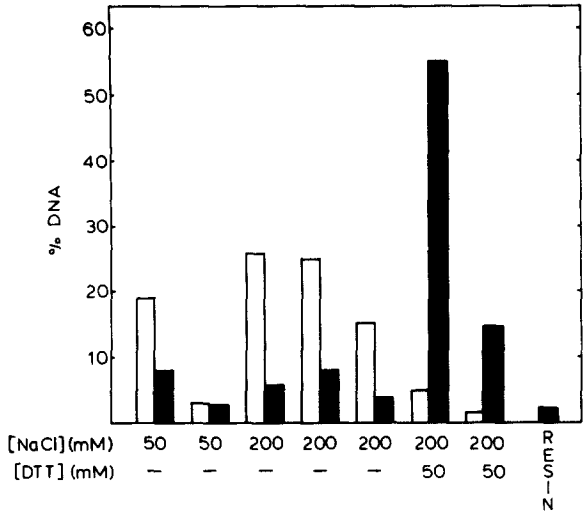

Figure 2. Avidin agarose affinity chromatography of nick translated DNA. DNA was purified from nuclei nick translated using 0.2 µg DNAse I per ml and applied to an avidin agarose column. The fraction of total DNA (open bars) or ^{3}H-labeled, nick translated DNA (solid bars) eluting from the column in each wash is indicated. The fraction of nick translated DNA remaining bound to the avidin agarose resin following the last wash is also indicated (resin).

fraction contained 70% of the nick translated, ^{3}H-labeled DNA (closed bars). Thus, nick translation of isolated nuclei with the chemically cleavable nucleotide, Bio-12-SS-dUTP, resulted in the affinity labeling of a small fraction of the total DNA which was subsequently isolated by affinity chromatography.

To determine wheather the affinity labeled DNA recovered from the avidin-agarose in DTT was enriched for transcriptionally active genes, the DTT washes were pooled and serial 2-fold dilutions of the DNA were applied to nitrocellulose paper and hybridized with a ^{32}P-labeled alpha^{D}-globin probe. The alpha^{D}-globin gene is known to exist in a DNase I sensitive conformation in adult erythrocyte nuclei (14). As shown in Figure 3, a significant enrichment of the alpha^{D}-globin gene was observed in the bound DNA fraction. Optimal enrichment of the globin gene in the bound fraction was obtained in the reaction containing 0.2 µg DNase I per ml. When nuclei were nicked with 1.0 µg DNase I per ml, the alpha^{D}-globin gene was almost completely digested (data not shown). Thus, a concentration of DNase I approximately 5-times lower than that resulting in the complete digestion of the gene is sufficient

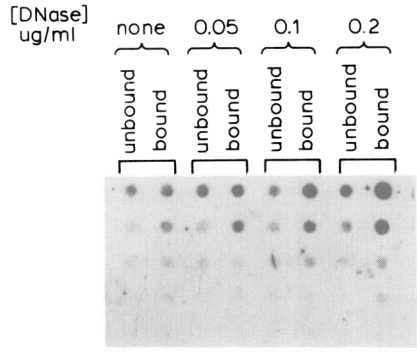

Figure 3. Dot hybridization of affinity purified DNA with an alpha^{D}-globin probe. Serial two-fold dilutions (beginning at 0.5 µg) of the unbound or bound DNA fractions were applied to nitrocellulose paper. The concentration of DNase I used in the nick translation reaction from which each DNA was purified is indicated.

to direct the affinity labeling of the alpha^{D}-globin gene by nuclear nick translation.

DISCUSSION

By including a chemically cleavable biotinylated nucleotide in the nuclear nick translation reaction first described by Levitt et. al. (2), we have shown that it is possible to affinity label a gene known to be in a transcriptionally active conformation of chromatin. DNA comprising this gene, and presumably other transcriptionally active genes, was enriched by avidin agarose affinity chromatography. Isolation was made possible by the chemically cleavable linker arm of the biotinylated nucleotide, Bio-12-SS-dUTP (7). We found that DNase I concentrations lower than those resulting in complete digestion of the alpha^{D}-globin gene are sufficient to direct the preferential labeling of this gene. Thus, it should be possible to isolate full length transcriptionally active genes by this procedure.

Hutchison and Weintraub (6) have recently suggested that only rapidly transcribed genes or genes that are maintained in a very active chromatin conformation are labeled by nuclear nick translation. Further studies comparing the enrichment of poorly or potentially transcribed genes with highly transcribed genes in our affinity labeled DNA fraction are underway to address this question. In addition, preliminary results indicate that this

procedure will also be useful in the affinity isolation of transcriptionally active regions of chromatin.

ACKNOWLEDGEMENTS

This work was supported in part by NIH grants AM34679-01 and HD20743-01 and a grant from the American Heart Association of Wisconsin (#85-GA-39). The technical assistance of Mary Hussin is gratefully acknowledged.

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