Localization of cytoplasmic-membrane-associated DNA in human chromosomes

(1H3rRNA/insituhybridization/chromosome 9/satellite DNA)

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ABSTRACT In situ hybridization was used to localize the sites of the repeated sequences of cytoplasmic-membrane-associated DNA (cmDNA) in human chromosomes. cmDNA was mainly present in the heterochromatic regions of chromosomes 9. Although cmDNA has buoyant densities in neutral and alkaline CsCl gradients similar to those of human satellite C DNA, which also is localized in the heterochromatic region of chromosomes 9, cmDNA was not homologous to satellite C DNA as determined by DNA-complementary RNA hybridization. These results are in agreement with our previous studies, which indicated that cmDNA is a unique species of human DNA.

Cytoplasmic-membrane-associated DNA (cmDNA), isolated from human lymphocytes, has been shown to possess physical and chemical properties which differ from those of chromosomal and mitochondrial DNAs (1). Reassociation kinetics of cmDNA show that this DNA is composed of two fractions with distinct reassociation rates (2). About 70% of cmDNA reassociates rapidly with a Cоt1/2 of 2.3 x 10^-4 mol-sec/liter and the remaining 30% reassociates with a Cоt1/2 of 223. The fast reassociating fraction is homologous to approximately 4% of the repetitive sequences of nuclear DNA, while the more slowly reassociating fraction hybridizes with 11% of the unique sequences of nuclear DNA (2).

It has been suggested that cmDNA is synthesized in the nucleus and subsequently “transported” to associate with cytoplasmic membranes (3). If cmDNA represents a selected class of sequences in the human genome, then chromosome localization of at least the repetitive portion of cmDNA may indicate its origin. Alternatively, cmDNA may contain repetitive sequences similar to those found in all chromosomes and give an ambiguous chromosomal localization pattern. In this report, localization was accomplished by employing the complementary RNA sequences synthesized from cmDNA templates in hybridization with metaphase chromosomes of human fibroblast cells and Wil2 cells.

MATERIALS AND METHODS

(a) Cell Culture and Cytological Preparation. Suspension cultures of Wil2 lymphocytes (4, 5) were grown in Auto-Pow BME medium supplemented with 10% fetal calf serum and other ingredients as described previously (2). Normal human fibroblasts initiated from human foreskin were obtained from Dr. T. R. Chen. Cells used for squashed slide preparations were grown in McCoy’s 5a medium supplemented with 20% fetal calf serum. Colcemid (0.01 µg/ml) was used to arrest the cells 2 hr before harvest.

(b) DNA Extraction and Fractionation. cmDNA was isolated from lysed cells as described previously (2). cmDNA was sheared to an average molecular weight of 2 x 10^6 and allowed to reassociate to a Cоt value of 5. The reassociated DNA, isolated by hydroxyapatite fractionation, is termed repetitive cmDNA (2). DNA was dialyzed against distilled H2O and then lyophilized. Complementary RNA was synthesized from DNA prepared in this manner. Satellite C DNA, isolated as described previously (6), was kindly provided by Dr. C. R. Chuang.

(c) Synthesis of Complementary RNA (cRNA). Radioactive RNA complementary to designated DNA fractions was synthesized using purified Escherichia coli DNA-dependent RNA polymerase as previously described (7). Synthesized cRNA was treated with DNase I ( Worthington, 10 µg/ml) for 30 min at 37°. Solutions were then extracted with an equal volume of redistilled phenol saturated with 2 X SSC (0.3 M sodium chloride–0.030 M sodium citrate, pH 7) followed by extraction with chloroform-isooamyl alcohol (24:1, vol/vol). The resulting aqueous phase was dialyzed against 2000 volumes of 2 X SSC overnight at 4°. The specific activity of cRNA was calculated to be 4.3 x 10^6 cpm/µg.

(d) In Situ Hybridization. The procedures used in these experiments were the same as described by Pardue and Gall (8). Autoradiographs were prepared by using Kodak thin film AR10 and developed in D19-B. The exposure time varied from 1 to 2 weeks unless otherwise indicated.

RESULTS AND DISCUSSION

When [3H]RNA complementary to native cmDNA is hybridized in situ with Wil2 cells or human fibroblast cells, the presence of reiterated sequences can be determined. In autoradiographs developed following a 2-week exposure, the silver grains were distributed on both interphase nuclei (not shown) and metaphase chromosomes (Fig. 1A). From 40 sets of duplicate photographs, each containing one photograph of the grain patterns and another of metaphase figures degrained for chromosome identification, all of the figures registered heavy clusters of silver grains over the constitutive heterochromatic regions of chromosomes 9. Grains were also over the constitutive heterochromatic regions of other chromosomes, but the density was not as spectacular as that found in chromosomes 9. Scattered single grains are seen on all chromosomes when the autoradiographs were overexposed (about 4 weeks). However, chromocenters always registered more grains than the diffuse chromatic area. Furthermore, autoradiographs of human nuclei show a somewhat uneven distribution (not shown).
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Previously we had shown that the heterochromatic areas of almost all human chromosomes are enriched in repetitive DNA sequences (9). This observation was confirmed using \[^{3}H\]RNA synthesized from repetitive DNA from Wil2 nuclei (data not shown). Thus the silver grain pattern of cRNA to repeat sequences of cmDNA is unlike that formed by cRNA to the total repetitive sequences of nuclear DNA in that repeat sequences of cmDNA are found primarily in chromosomes 9.

At first glance, cmDNA has characteristics similar to several other defined human species. For example, the buoyant densities in neutral CsCl of satellites C (\(\rho = 1.703 \text{ g/ml}\)) and III (\(\rho = 1.699 \text{ g/ml}\)) and cmDNA (\(\rho = 1.699 \text{ g/ml}\)) are similar. In \textit{in situ} hybridization experiments show very similar chromosome localization patterns for satellites C and III and cmDNA. However, their densities in alkaline CsCl are different: satellite C, \(\rho_{\text{alk}} = 1.760 \text{ g/ml}\); satellite III, \(\rho^{\text{light}}_{\text{alk}} = 1.722 \text{ g/ml}\) and \(\rho^{\text{heavy}}_{\text{alk}} = 1.759 \text{ g/ml}\); cmDNA \(\rho_{\text{alk}} = 1.756 \text{ g/ml}\). When centrifuged together with them in a CsCl density gradient, satellite C was clearly differentiated from the other two. The buoyant density values shown here for satellite III were obtained in our laboratory (10) and contrast with those reported by Corneo et al. (11): \(\rho_{\text{nucl}} = 1.696 \text{ g/ml}\); \(\rho^{\text{light}}_{\text{alk}} = 1.740 \text{ g/ml}\); \(\rho^{\text{heavy}}_{\text{alk}} = 1.754 \text{ g/ml}\).

Sequence homology between satellite C and cmDNA was measured by hybridization of satellite C transcripts to immobilized denatured Wil2 nuclear DNA and cmDNA. Under the conditions used in these experiments, similarity in base sequence in the repetitive regions can be determined. The data presented in Table 1 show that two to three times more \[^{3}H\]cRNA became RNase resistant in hybrids formed with nuclear DNA than with cmDNA. Since cmDNA is homologous with 4% of nuclear repetitive DNA and the repetitive sequences constitute 35% of total nuclear DNA, then the amounts of homologous repetitive DNA sequences were: (a) nuclear DNA, 0.04 \times 0.35 \times 20 \mu g/filter = 0.28 \mu g; (b) cmDNA, 0.70 \times 20 \mu g/filter = 14 \mu g. Thus, 50 times more hybrid should have been formed if cmDNA and satellite C contained sequence homology in their repetitive sequences. Therefore, the finding that RNA complementary to satellite C DNA bound preferentially to nuclear DNA indicates that satellite C DNA is not related to cmDNA despite their similar locations in chromosomes 9 and their similar buoyant densities in neutral and alkaline CsCl gradients.

The finding that repeated sequences of cmDNA are primarily located in the constitutive heterochromatic region of chromosomes 9 confirms our previous observation that cmDNA represents a selected class of sequences (2). The location of repetitive cmDNA sequences in chromosomes 9 is interesting since other unique DNA species, namely satellites C and III, have similar chromosome locations. Furthermore, such chromosome localization tends to confirm our previous notion that cmDNA is synthesized in the nucleus.

Table 1. Hybridization of satellite C cRNA to human nuclear DNA and cmDNA

<table>
<thead>
<tr>
<th>DNA on filters*</th>
<th>(^{3}H) cpm bound (\dagger)</th>
<th>(^{3}H) cpm bound (\ddagger)</th>
<th>(^{3}H) cpm bound minus control (\ddagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Salmon sperm</td>
<td>9,633</td>
<td>0.72</td>
<td>0</td>
</tr>
<tr>
<td>Nuclear</td>
<td>34,559</td>
<td>2.59</td>
<td>1.87</td>
</tr>
<tr>
<td>cmDNA</td>
<td>13,316</td>
<td>1.00</td>
<td>0.28</td>
</tr>
<tr>
<td>2. Salmon sperm</td>
<td>8,357</td>
<td>0.63</td>
<td>0</td>
</tr>
<tr>
<td>Nuclear</td>
<td>37,723</td>
<td>2.82</td>
<td>2.19</td>
</tr>
<tr>
<td>cmDNA</td>
<td>16,308</td>
<td>1.22</td>
<td>0.59</td>
</tr>
<tr>
<td>3. Salmon sperm</td>
<td>6,511</td>
<td>0.49</td>
<td>0</td>
</tr>
<tr>
<td>Nuclear</td>
<td>38,523</td>
<td>2.88</td>
<td>2.39</td>
</tr>
<tr>
<td>cmDNA</td>
<td>13,517</td>
<td>1.01</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* 20 \mu g/filter. Incubation was at 37\(^\circ\) for 24 hr in 2 \times SSC. After incubation, filters were washed (with suction) with 2 \times SSC, treated with RNase for 1 hr at 37\(^\circ\), and washed again with 2 \times SSC (50 ml of 2 \times SSC on each side). Numbers refer to each reaction vial containing the three DNA-filters.

\(\dagger\) Total \(^{3}H\) cpm in each reaction vial was 1,335,400 cpm.

\(\ddagger\) Control is \(^{3}H\) cpm bound to salmon sperm DNA.
prior to its appearance on cytoplasmic membranes (3). Finally, the appearance of a selected class of nuclear DNA sequences on cytoplasmic membranes (1-3) supports the notion that this DNA has some biological role.

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