Facioscapulohumeral muscular dystrophy (FSHD) is located on chromosome 4q35, close to the telomere. FSHD patients carry deletions within a cluster of tandemly repeated DNA. Although expression of a functional FSHD gene will be altered in patients, the sequence itself may be unaffected by this deletion. Hence, the FSHD gene could lie outside of the deleted region. This study employs fluorescent in situ hybridization using chromosome 4-specific cosmids and YAC clones to rapidly saturate chromosome 4 with new markers. Some 250 cosmids and 26 YACs were regionally mapped, of which 5 YACs and 55 cosmids mapped to the distal portion of 4q. Only one of these clones (D4S1454) mapped telomERICally to a translocation breakpoint specified by D4S187. Using two-color interphase mapping, the following marker order was obtained: Cen–D4S187–D4S1454–HSPCAL2–D4S163–D4S139–D4F35S1. Absence of additional markers mapping distal to D4F35S1 indicates that the linkage group containing the FSHD gene lies extremely close to the 4q telomere. © 1995 John Wiley & Sons, Inc.

Key Words: facioscapulohumeral muscular dystrophy (FSHD) • chromosome 4 • fluorescent in situ hybridization • cosmids • YACs

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proper, or exons of the gene, are unlikely to reside within the 3.3-kb repeat. Consequently, our working hypothesis is that the gene must lie either proximal or distal to the repeat cluster. For two reasons a relatively large area needs to be included in the gene search. First, deletions within the tandem repeat may cause a position effect that changes the expression of an otherwise intact FSHD gene by altering the chromatin structure. The distances over which such position effects can extend are completely unknown. Second, genes can show an enormous size variation. For instance, in the case of the dystrophin gene, introns as large as a few hundred kilobases have been found. Both arguments favor a gene search in a very large region surrounding the D4F104S1 locus.

The aim of this study is to augment the existing marker set around the FSHD locus with new cosmid and YAC clones. Of particular interest is the search for clones mapping distal to cosmid 13E, since no YAC or cosmid clones have been identified so far that extend more telomerically. We have used fluorescent in situ hybridization (FISH), which is a powerful technique to rapidly localize cosmid or YAC clones. Three approaches were applied. First, clones were mapped with relatively low resolution to metaphase chromosomes. Second, a more defined localization was obtained by mapping clones relative to two different translocation breakpoints in the FSHD gene region. Third, the use of different colors as fluorescent label makes it possible to perform hybridization of multiple clones to interphase nuclei simultaneously. This allowed us to order clones relative to each other.

MATERIALS AND METHODS

Cell Lines. Cell line GM11025 (Human Genetic Cell Repository, Camden, NJ) contains a translocation t(X;4) (p21;q35), in which the chromosome 4 breakpoint corresponds with the locus D4S187. The exact position of the GM2346 breakpoint is unknown. Cosmid 13E, which contains D4F104S1, recognizes sequences at both sides of the translocation breakpoint.

Cosmids.
A chromosome 4-specific cosmid library (Los Alamos Chromosome 4 Library) was gridded out on 96-well micrometer dishes and, in addition, plated out on filters (5000 colonies/plate). Cosmids were either randomly selected from the gridded library, or isolated by colony hybridization with specific probes for the loci D4S163 (LILA5), D4S139 (pH30), and D4F35S1 (CEB8). A cosmid containing the heat shock protein 90α gene (HSPCAL2) was a kind gift of Dr. Yokayama (Tsukuba, Japan).

Fluorescent In Situ Hybridization (FISH). Metaphase spreads were prepared according to standard protocols. Total DNA of either cosmid or YAC clones was nick translated using the BioNick labeling system (BRL). After purification on a Sephadex-G50 fine column, the DNA was precipitated in the presence of 25-fold excess of human placental DNA (sonicated to 500-bp average size), or in cases of extremely repetitive probes, with Cot1 DNA. Hybridization and detection of cosmid and YAC probes was performed according to Kievits et al. and Driesen et al., respectively. Chromosomes were stained with 4′,6-diamidino-2-phenylindole.2HCl and actinomycin D.

Yeast Artificial Chromosomes (YACs). From a chromosome 4-specific YAC library, constructed from the mouse–human hybrid cell line HA(4)A, 34 YAC clones were selected with an insert size larger than 100 kb. Yeast colonies were inoculated overnight in 3 mL of synthetic minimal (SD) medium. Yeast DNA was isolated according to Green and Olson.

FIGURE 1. Relative positions of cell lines GM11025 (t(X;4)) and GM2346 (t(4;16)) to the existing linkage group on 4q35. The breakpoint in cell line GM11025 corresponds to locus D4S187. The exact position of the GM2346 breakpoint is unknown. Cosmid 13E, which contains D4F104S1, recognizes sequences at both sides of the translocation breakpoint.
All probes were first hybridized to a normal human karyotype metaphase, and assigned to ten regions of chromosome 4. In addition, the clones assigned to the 4q32-pter region were hybridized with respect to the translocation breakpoints of the cell lines GM11025 and GM2346. To establish the order of the probes that map in between the two breakpoints, interphase mapping was performed. Cosmid DNA was labeled either in the presence of biotin-11-dUTP, or dioxigenin-11-dUTP. For the human karyotype metaphase, and assigned to ten regions of chromosome 4 were hybridized to this breakpoint. The localization of this cosmid corresponds to the locus D4S187.

**RESULTS**

From a total of 250 of the 315 randomly selected cosmids (80%), from 26 YAC clones of the 34 (76%), a specific hybridization signal was observed. None of the YAC clones showed hybridization to another chromosome than chromosome 4. The approximate location of these 276 clones was established by estimating its relative position between the centromere and the telomere. The short arm was divided into three sections, whereas the long arm was divided into seven sections. We did not use reference points or simultaneous R- and Q-banding of the metaphase spreads. Clones could easily be assigned to the distal part of the long arm or the short arm, and at both sides of the centromere. However, the mapping of clones in between these areas is less precise. The FISH mapping data are summarized in Table 1.

The mapped clones are more or less evenly distributed throughout chromosome 4. Every section receives approximately 10% of the clones, with the exception of the region on 4p closest to the centromere (6.1%), and the most telomeric region of 4q (16.3%). An estimate based on the appearance of prometaphase chromosomes shows that 4p is about one third of the size of 4q. The results of this study agree with this estimate, since 72 clones (76%), a specific hybridization signal was observed, and YAC clones assigned to 10 regions on chromosome 4.

<table>
<thead>
<tr>
<th>Table 1. Randomly selected chromosome 4-specific cosmid and YAC clones assigned to 10 regions on chromosome 4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4p15.3-p14 S722, S723, S724, S725, S726, S727, S728, S729, S730, S732, S733, S734, S735, S736, S737, S738, S739, S740, S741, S743, S1400, S1401, S1402, S1403, S1404, S1465 (125 kb), S1466 (130 kb)</td>
</tr>
<tr>
<td>4p14-p11 S744, S745, S746, S747, S748, S749, S750, S751, S752, S754, S755, S756, S757, S1405, S1467 (210 kb), S1468 (120 kb), S1469 (160 kb)</td>
</tr>
<tr>
<td>4q22-q26 S626, S627, S628, S629, S630, S631, S632, S633, S634, S635, S805, S806, S807, S1423, S1424, S1425, S1426, S1427, S1428</td>
</tr>
<tr>
<td>4q26-q28 S637, S638, S639, S640, S641, S642, S643, S645, S646, S648, S649, S650, S651, S652, S653, S1429, S1430, S1431, S1432, S1433, S1434, S1474 (260 kb), S1475 (140 kb), S1476 (135 kb)</td>
</tr>
<tr>
<td>4q28-q31.2 S654, S655, S656, S657, S659, S660, S661, S662, S663, S664, S665, S666, S667, S668, S670, S671, S1436, S1437, S1438, S1439, S1440, S1441, S1442, S1443, S1444</td>
</tr>
<tr>
<td>4q31.2-q32 S672, S673, S674, S675, S676, S677, S678, S679, S681, S682, S683, S684, S685, S686, S687, S688, S1444, S1445, S1446, S1447, S1479 (195 kb), S1480 (125 kb), S1481 (110 kb), S1482 (105 kb), S1483 (150 kb)</td>
</tr>
<tr>
<td>4q35 S1484 (100 kb), S1485 (105 kb), S1486 (120 kb), S1487 (110 kb), S1488 (160 kb)</td>
</tr>
</tbody>
</table>

*The sizes of the individual YAC clones are given in parentheses.*
Table 2. Interphase mapping.

<table>
<thead>
<tr>
<th>Cosmids</th>
<th>Observation</th>
<th>Locus order</th>
</tr>
</thead>
<tbody>
<tr>
<td>D4S139, D4S163, D4S1454</td>
<td>G-R-R</td>
<td>D4S1454—D4S139—D4S163</td>
</tr>
<tr>
<td>D4F35S1, D4S139, D4S1454</td>
<td>G-G-R</td>
<td>D4S1454—D4S139—D4F35S1</td>
</tr>
<tr>
<td>D4S1454, D4S139, D4S163, D4F35S1</td>
<td>G-R-G</td>
<td>D4S1454—D4S139—D4F35S1</td>
</tr>
<tr>
<td>D4F35S1, D4S139, D4S1454</td>
<td>G-R-G</td>
<td>D4S1454—D4S139—D4S163</td>
</tr>
<tr>
<td>D4F35S1, D4S139, D4S163, D4S1454</td>
<td>G-R-G-R</td>
<td>D4S1454—D4S139—D4F35S1</td>
</tr>
</tbody>
</table>

Cosmid DNA was labeled to give a green FITC fluorescence (G) or a Texas Red fluorescence (R). The cosmids that have been labeled with Texas Red are underlined.

(4cen...HSPCAL2—D4S163—D4S139—D4F35S1—D4F104S1...4qter)\(^{24,29,31,32}\) was established using interphase mapping with combinations of different cosmids in two colors (Table 2). These data indicate that D4S1454 maps between D4S187 and HSPCAL2.

DISCUSSION

We used FISH to sublocalize 250 cosmid clones and 26 YAC clones to chromosome 4. A random distribution of all the FISH mapped clones along chromosome 4 with a predicted length of 200 Mb\(^{14}\) implies that we have mapped one clone approximately every 700 kb. The fact that only one cosmid was found to map distal to the breakpoint in GM11025 could imply that there is not more than about 1–2 Mb of DNA distal to D4S187. However, the genetic distance between D4S187 and the FSHD gene is about 10 cM\(^{25,26}\), i.e., 5–10 times more than expected from the above calculation. Based on contig mapping using YACs, the region D4S187–D4F104S1/FSHD is estimated to be 2.4 Mb in length\(^{24}\), which suggests a fourfold increased genetic distance compared to the physical one. Such increased recombination frequencies are thought to be part of the general behavior of subtelomeric regions\(^{2,20}\) and might explain the discrepancy found between the genetic and physical distance at 4qter.

Unfortunately, we did not succeed in the isolation of YAC or cosmid clones mapping distal to D4F104S1. The reasons for this phenoma could be that the region is very small and/or consists of unclonable DNA. Based on FISH experiments using interphase chromosomes, the 4q telomere is demonstrated to be within 215 kb of the tandem array of 3.2-kb repeat units\(^{30}\). Since the 4q telomere seems to be in close proximity to the tandem repeats, it is not unlikely that the region distal to these repeats consists of telomere-associated repeats, which might be extremely difficult to clone. Yet we cannot exclude that the FSHD gene resides in this most distal part of chromosome 4q. However, due to the absence of telomeric material, the present gene search focuses on the proximal region between D4S139 and D4F104S1. We were able to isolate a new cosmid (D4S1454) that maps within the FSHD linkage group. Based on the YAC contigs in this region\(^{27}\), this cosmids maps at least 1 Mb proximal to D4F104S1.

This study shows that FISH mapping can be a valuable complementary strategy for the detailed mapping of a limited chromosomal region. The ordering of clones in interphase nuclei by using two different fluorophores demonstrates the high resolving power of this approach. In comparison to several other techniques, FISH is very efficient, rapid, and easy. Within 2 days, 20–30 cosmid or YAC clones can be regionally localized. Therefore, these randomly distributed clones may provide a good starting resource for investigators interested in obtaining probes that span particular translocation or deletion breakpoints.

REFERENCES


