High-Resolution Chromosome Sorting and DNA Spot-Blot Analysis Assign McArdle's Syndrome to Chromosome 11

Abstract. *A rapid gene-mapping system uses a high-resolution, dual-laser sorter to identify genes from separate human chromosomes prepared with a new stain combination. This system was used to sort 21 unique chromosome types onto nitrocellulose filter papers. Several labeled gene probes hybridized to the sorted chromosomal DNA types predicted by their previous chromosome assignments. The skeletal muscle glycogen phosphorylase gene was then mapped to a portion of chromosome 11 by spot blotting normal and translocated chromosomes.*

Chromosome sorting, a powerful technique for studying human genetics (*1, 2*), has been used for karyotype analysis (*3*), structural gene mapping (*4*), and construction of chromosome-specific recombinant DNA libraries (*5*). The procedure reported earlier to map genes by chromosome sorting (*4*) only separated 12 chromosome fractions and required long sorting times to isolate 2 million chromosomes for restriction enzyme analysis. Improvements in single-laser chromosome analysis revealed 15 to 20 fractions with 9 to 14 individual chromosome types (*6*), and dual-laser analysis resolved 18 fractions with 14 single types (*7*). We now describe our improved dual-laser method (*8*) and a new stain combination, DIPI[1]-chromomycin (*9*), which we used to sort and identify 22 chromosome fractions with 21 unique types. Thirty thousand chromosomes of each type were sorted directly onto nitrocellulose filters to avoid DNA extraction. A panel of filters (spot blots) was sorted in 2 days, and each panel could be used five times to map genes (*10*). We localized the gene-encoding skeletal muscle glycogen phosphorylase (myophosphorylase) (E.C. 2.4.1.1) to chromosome 11. A defect in this gene product results in McArdle's syndrome (Glycogenosis Type V) (*11*).

Normal chromosomes exhibit polymorphic banding patterns (*12*). Thus, two homologous chromosomes may have different total fluorescence and may be separated by flow-sorting analysis. This often results in one homologous chromosome contaminating a neighboring fraction that contains another chromosome type. We therefore tested several permanent human lymphocyte cell lines to characterize the karyotypically normal cell line GM130 (*13*), from which homologous chromosomes are not only superimposed in their total fluorescence but are also well separated from other chromosome types. All chromosomes stained with Hoechst-chromomycin from this cell line, except chromosomes 9, 10, 11, and 12, were resolved into separate fractions (Fig. 1A).

During a survey of DNA-specific dyes we found that the chromosomes 9, 10, 11, and 12 could be resolved further when stained with DIPI-chromomycin. Comparison of our karyotypes of Hoechst-stained with DIPI-stained GM130 chromosomes revealed that chromosomes 1, 9, 15, 16, and Y each had more brightly staining regions with DIPI (data not shown). This was consistent with reports that the same chromosome regions remained brightly fluorescent when stained with DIPI and counterstained with distamycin A (*9, 14*). Flow-sorting analysis of chromosomes stained with DIPI-chromomycin from GM130 separated chromosome 9 from chromosomes 10, 11, and 12 and further separated chromosomes 16 and 17 (Fig. 1).

We showed that chromosomes prepared and sorted in tris-spermine buffer (*6*) could be identified by quinacrine-banding analysis (legend to Fig. 2). In this manner we identified and ascertained the purity of chromosomes sorted with each stain pair. Except for chromosomes 8 and 9, 95 to 99 percent of the chromosomes sorted in each fraction were of the type labeled for each histogram peak (Fig. 1). The chromosome 8 and 9 fractions were 85 percent pure, with the major contaminant being the other chromosome.

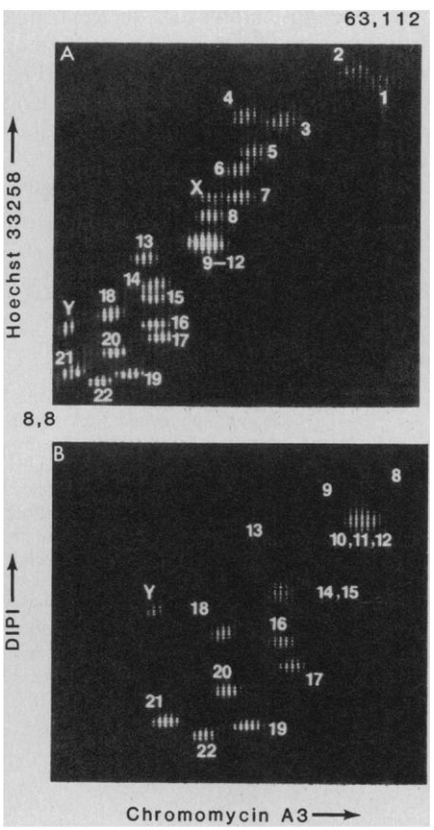

Fig. 1. Flow histograms of normal human male chromosomes (GM130). Portions of channel histograms (64 by 128) of flow-analyzed lymphocyte chromosomes viewed from above a three-dimensional histogram. The two laser-excited fluorescence signals were measured separately and correspond to chromomycin fluorescence from the first laser in the *x*-axis and to DIPI or Hoechst fluorescence from the second laser in the *y*-axis. The light intensity of each dot in the three-dimensional histogram is linearly proportional to the number of chromosomes recorded in each channel. (A) All chromosomes stained with Hoechst and chromomycin A3. (B) Chromosomes 8 to 22 and Y stained with DIPI and chromomycin. Data were acquired without gating or mathematical manipulation. Chromosomes prepared in tris-spermine buffer (*14*) were stained with 0.4 μg of DIPI per milliliter (*9*) or with 0.4 μg of Hoechst 33258 per milliliter (Polysciences) and 4 μg of chromomycin A3 per milliliter (Sigma) and were analyzed and sorted (*8*). Chromosomes to be identified by quinacrine-banding were sorted into a warm Lief bucket, fixed immediately, and then centrifuged (*2, 4*).

This system of separating and identifying human chromosomes enabled us to develop a rapid method of assigning cloned human genes to specific chromosomes. Thirty thousand chromosomes of each type, representing 3 to 15 ng of DNA, were sorted directly onto nitrocellulose filters. The chromosomal DNA was denatured and tested with ^{32}P-labeled unique DNA probe as for restriction enzyme analysis (*4*). To verify the validity of the spot-blot method, we tested several genes that had been mapped earlier. The ζ-globin probe hybridized to the spot containing chromosome 16 (Fig. 2), the β-globin probe to chromosomes 10, 11, and 12, and the human cellular *sarc* oncogene to chromosome 20 (data not shown), in agreement with other gene mapping data (*4, 6, 15*).

We then used this method to determine the unknown chromosomal location of the muscle myophosphorylase gene, which is deficient in McArdle's syndrome (*11*). The carboxyl-terminal region of the myophosphorylase gene region was isolated from a bacteriophage lambda Charon 4a human genomic library (*16*). Partial sequencing of this gene fragment revealed that the codons for amino acids 732 to 840 and the 3′ untranslated region were identical to the

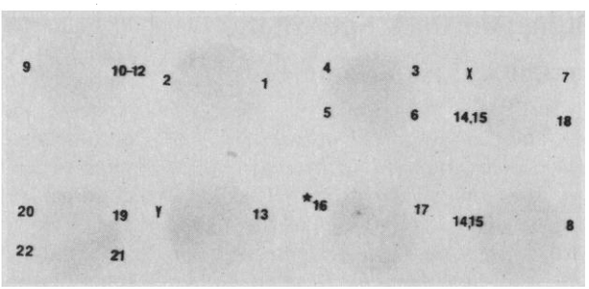

Fig. 2. Autoradiograph of dot-blot filters. An autoradiograph of a panel of filters (25 mm in diameter) hybridized to nick-translated ϵ-globin gene probe showed a gene-specific signal in the fraction containing chromosome 16. A spot-blot panel was constructed from chromosome preparations made with 4×10^7 cells in 200 ml of lymphocyte suspension culture. Chromosomes for spot-blot analyses were sorted directly onto a nitrocellulose filter disk (25 mm in diameter, 0.45 μm pore size; Millipore) and were placed on a scintered glass Millipore filter (25 mm in diameter) under vacuum. The sorted chromosomal DNA was denatured for 5 minutes with 0.5*N* NaOH and 1.5*M* NaCl, neutralized with 0.5*M* tris-HCl (*p*H 7.5), 3*M* NaCl (three 5-minute washes), and hybridized to a ^{32}P-labeled probe (2×10^8 to 10×10^8 count/min per microgram).

sequence of a complementary DNA (cDNA) clone encoding myophosphorylase that was obtained from a human muscle cDNA library (*16*). When a filter set that had been used earlier was hybridized to a nick-translated carboxyl-terminal gene fragment, the gene-specific signal appeared in the spot containing chromosomes 10, 11, and 12.

To determine on which of these three chromosomes the myophosphorylase gene resides, we sorted chromosomes from cell lines in which translocations affecting these chromosomes would move one affected homologous chromosome away from chromosomes 10, 11, and 12. We first tested two chromosomes in cell line GM5519 (*13*) generated by a reciprocal translocation between the short arm of chromosome 11 and the long arm of chromosome 4, which are intermediate in size (Fig. 3A). The derivative chromosome 11 [der(11)], which contains the long arm, centromere, and proximal portion of the short arm of chromosome 11, was sorted with chromosomes 5 and 6, while der(4), which contains the rest of the short arm of chromosome 11, was sorted with chromosomes X and 8 (not shown). Spot-blot tests of these chromosome fractions revealed the muscle phosphorylase gene in the fraction containing der(11) (Fig. 3B), thus assigning the gene to chromosome 11 (11p13 → 11qter) and excluding it from the distal portion of the short arm.

To further localize the myophosphorylase gene, we tested a series of Chinese hamster–human somatic cell hybrid DNA's that had been constructed earlier and contained a single human chromosome 11 with various terminal deletions (*17*). Southern blot analysis (*18*) revealed the presence of the muscle phosphorylase gene in hybrids J1-7 and J1-8 (Fig. 3A), which contained an intact long arm of chromosome 11 with variable short arm deletions. However, the gene was lost when the distal portion of the long arm was deleted in hybrid J1-11 (Fig. 3A), thus assigning the gene to the long arm of chromosome 11 (q13 → qter) (data not shown).

The spot-blot gene mapping method is efficient for several reasons. First, a filter panel with all the chromosomes may be sorted in only 2 days. Second, chromosomal DNA is tested directly, without DNA extraction or restriction enzyme digestion. Third, fewer chromosomes (30,000) are required to detect individual gene-probe hybridization. Fourth, we have improved chromosome yield and identified sorted chromosomes by using (i) a suspension cell line instead of fibroblasts and (ii) spermine buffer (*6*) to reduce random and centromeric chromosome breakage. Finally, filter panels that are constructed earlier may be tested and retested rapidly to map up to five DNA probes. Chromosome sorting has been used to identify a Y-specific sequence (*19*). However, by using a dual laser sorter and improved methods, we are now able to map a gene to any one of 21 human chromosomes in a single hybridization step. A gene mapped to the fraction containing chromosomes 10, 11, and 12 may be localized subchromosomally by testing derivative chromosomes (*2*, *13*).

Somatic cell hybridization has been used to map genes by testing protein expression and, more recently, by DNA hybridization to cloned gene probes (*20*). This spot-blot method has significant advantages for testing cloned gene probes. Spot blotting requires a single permanent cell line with a stable normal chromosome complement. In contrast, unstable chromosome constitutions of somatic cell hybrid panels must be verified by repeated karyotyping. In addition, in some hybrid cell lines human chromosome fragments move to rodent chromosomes, where they are not detected by karyotyping. For instance, the human insulin gene remained in the J1 somatic cell hybrids (*21*) when all identifiable human chromosomes were lost (*17*). Spot blotting can map genes regionally by sorting both derivative chromosomes and their normal homologous counterparts in 1 hour. Considerably more time is required to construct and karyotype new somatic cell hybrids.

This spot-blotting method can be used to locate genes subchromosomally to the resolution of the derivative chromosomes available. Studying early metaphase and prometaphase karyotypes on in situ slides (*22*) can improve this resolution, but scoring each chromosome in each karyotype is tedious. A complementary procedure would be to map a gene to one chromosome by spot blotting and then to locate the gene regionally by scoring only the chromosome of interest on the slide developed in situ.

These improvements in chromosome

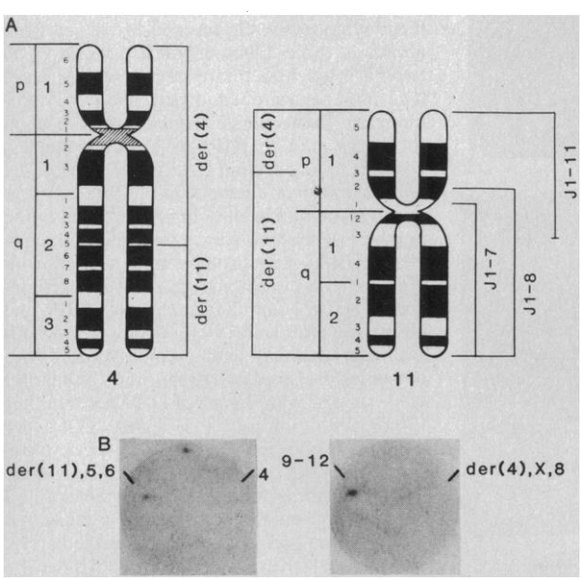

Fig. 3. Sublocalization of muscle phosphorylase. (A) Idiograms indicate the chromosome regions reciprocally translocated between the short arm of chromosome 11 and the long arm of chromosome 4. The two derivative chromosomes are intermediate in size. (B) Spot-blot analysis revealed that der(11) and the fraction containing the normal chromosome 11 each contained muscle phosphorylase sequences.

staining, sorting, and gene mapping will be useful (i) to locate random DNA fragments for linkage analysis with human disease loci and (ii) to improve flow karyotype diagnoses. Improved chromosome resolution will separate normal and abnormal chromosomes with increased purity to construct libraries from which linked DNA fragments can be chosen. The spot-blot method can then be used to determine the purity and subchromosomal location of any library fragment. Comparison of flow karyotypes of chromosomes stained with DIPI-chromomycin with those stained with Hoechst-chromomycin will detect differences in polymorphic chromosome bands to distinguish more reliably normal polymorphic variability from abnormal chromosomes.

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Selective Tropism of Lymphadenopathy Associated Virus (LAV) for Helper-Inducer T Lymphocytes

Abstract. *Lymphadenopathy associated virus (LAV) has been isolated from patients with the acquired immunodeficiency syndrome (AIDS) or lymphadenopathy syndrome. Since the immune deficiency in AIDS seems to be primarily related to the defect of the helper-inducer T lymphocyte subset, the possibility that LAV is selectively tropic for this subset was investigated. Fractionation of T lymphocytes was achieved by cellular affinity chromatography with monoclonal antibodies. In a hemophilic patient who was a healthy carrier of LAV, reverse transcriptase activity and virus particles detected by electron microscopy were found only in cultures of helper-inducer lymphocytes. When infected with LAV in vitro, lymphocyte subsets from normal individuals yielded similar results. Virus production was associated with impaired proliferation, modulation of T3-T4 cell markers, and the appearance of cytopathic effects. The results provide evidence for the involvement of LAV in AIDS.*

Epidemiological data indicate that the acquired immunodeficiency syndrome (AIDS) is caused by an infectious agent, probably a virus (*1–3*). Since a qualitative and quantitative defect of the helper-inducer T cell subset ($T4^+$) is the major immunological abnormality of this disease (*4, 5*), one would expect such a causative agent to express a tropism for $T4^+$ lymphocytes, resulting in alteration of their function.

We recently discovered a new group of human retroviruses that differ from human T-cell leukemia virus (HTLV-I) (*6–10*). The first member of this group was isolated from a nonimmunodepressed homosexual patient with persistent lymphadenopathy, a syndrome considered to be related to AIDS (*11, 12*). This virus has tentatively been named lymphadenopathy associated virus (LAV). Similar viruses were found in patients with frank AIDS, including the only hemophiliac patient with AIDS reported in France (*7*). Specific antibodies against LAV have been detected in approximately 70 percent of patients with persistent lymphadenopathy and 40 percent of AIDS patients studied (*8, 13*). We have now investigated whether the tropism of LAV is selective for the $T4^+$ subset in vivo as well as in vitro.

The studies in vivo were conducted with the help of an asymptomatic virus carrier, patient E.L., and his brother, patient D.L. Both patients have B hemophilia (*7*). Patient D.L. recently developed AIDS as assessed by successive opportunistic infections accompanied by a profound alteration of immune functions in vivo and in vitro. By means of techniques already described (*6–8*), a retrovirus similar to LAV was isolated repeatedly from his peripheral blood lymphocytes (PBL). This virus differs from HTLV-I (*9, 14, 15*). When examined by an immunofluorescence technique with antibodies to the structural proteins p24 or p19 of HTLV-I, fixed LAV-producing cells (*6*) were negative. Similarly, [^{35}S]methionine-labeled p25 of LAV was not precipitated by the antibodies to HTLV-I. Moreover, no antibody to HTLV-producing cell lines (C10/MJ, C91/PL) could be detected in the patient's serum by immunofluorescence, nor to HTLV p24 by the commercial enzyme-linked immunosorbent assay (ELISA; Biotech) or by a radioimmunoassay with a monoclonal antibody to p24 (*7*). No antibodies against LAV or HTLV could be detected in serum samples from parents of the patients, but E.L., like his brother, had circulating antibodies against LAV. However, apart from having hemophilia, E.L. was healthy and displayed normal immune functions. Nevertheless, LAV was isolated three times at monthly intervals from his PBL. The fact that no difference could be found between the viruses isolated from the brothers by serological and morphological studies, as well as the similar history of factor IX transfusion,