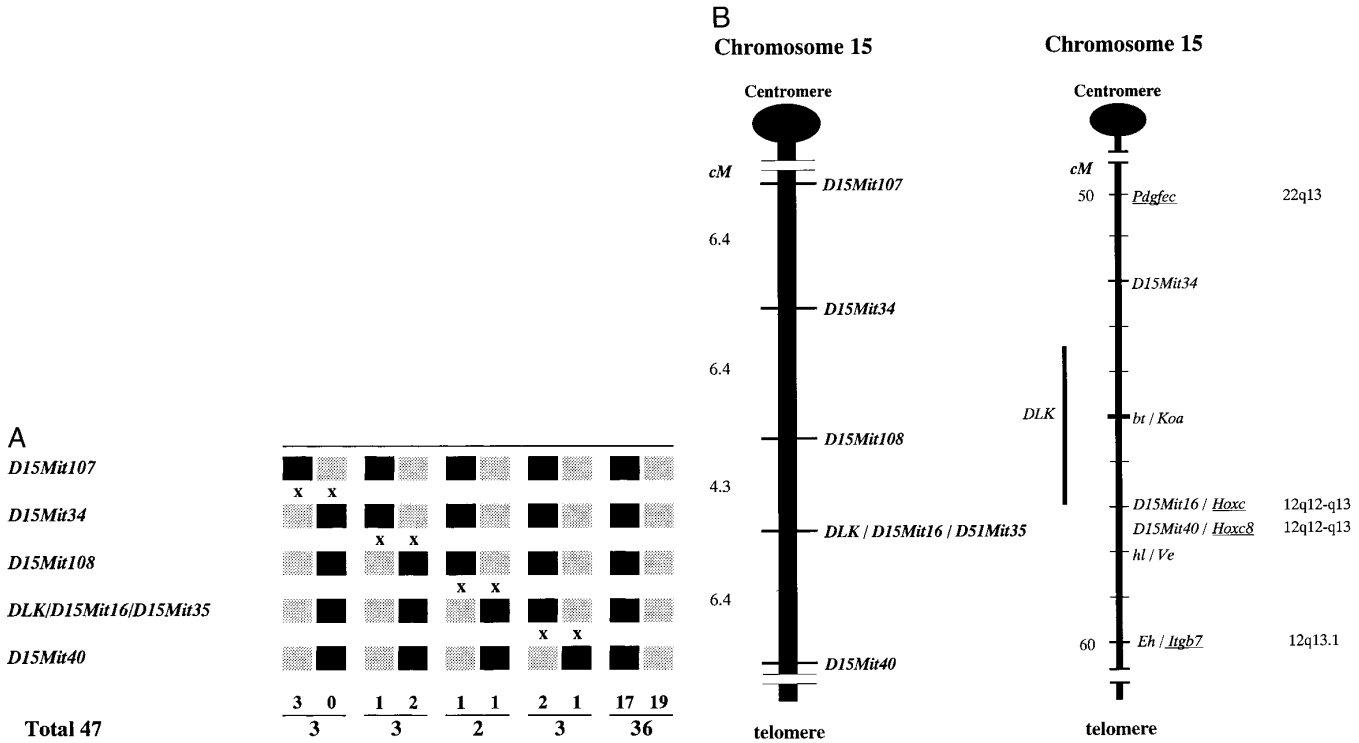

FIG. 1. Chromosomal localization of mouse *DLK*. (**A**) Haplotype analysis of the interspecific backcross progeny carrying recombinants between *D15Mit107* and *D15Mit40*. Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6 × *Mus spretus*) F1 parent. The black squares represent the presence of both the *Mus spretus* and the C57BL/6 alleles and the stippled squares the presence of either the C57BL/6 or the *Mus spretus* allele. Locus order was determined by minimizing the number of observed recombination breakpoints. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. (**B**) Linkage map of the proximal region of mouse Chromosome 15 constructed based on the analysis of the interspecific backcross (**left**) or on the Mouse Genome Database (MGD). Genetic distances are given in centimorgans and shown for each pair of loci (**left**) or from the centromere (**right**) on the left of each chromosome map. The positions of the underlined loci in the human chromosome are shown to the right of the chromosome map. References for the human map positions of loci cited in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, MD).

A PAC Containing the Human Mitochondrial DNA Polymerase Gamma Gene (*POLG*) Maps to Chromosome 15q25

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Received August 21, 1996; accepted December 20, 1996

The human mitochondrial DNA (mtDNA) is a closed circular, 16,569-bp double-stranded DNA, encoding 13 genes whose protein products are subunits of the oxidative phosphorylation system required for synthesis of most of the ATP consumed by eukaryotic cells. Point mutations of the mtDNA that cause multi-tissue, loss-of-energy syndromes, called mitochondrial encephalomyopathies (e.g., MERRF and MELAS), have been identified. In addition, large-scale deletions of the human mtDNA have been identified and are the molecular bases for the neonatal and adolescent onset loss-of-energy syndromes Pearson and Kearns–Sayer, respectively (3, 5).

An intriguing question regarding human mitochondrial loss-of-energy disorders is the extent to which genetic predisposition controls the age-related onset of bioenergetic mutations on the human mtDNA. Traditionally, oxidative stress has been implicated as a major influence in causing point mutations of the human mtDNA (5). Support for this notion is the age-related increase in mtDNA base modifications, especially 8-hydroxydeoxyguanosine in brain and heart, which may lead to somatic mtDNA point mutations. Large-scale deletions of the human mtDNA are detected in oxidative tissues of the elderly and also appear to be somatic. The mechanism of mtDNA deletion is unknown, but it may be initiated

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GENOMICS **40,** 376–378 (1997)
ARTICLE NO. GE964580
0888-7543/97 $25.00

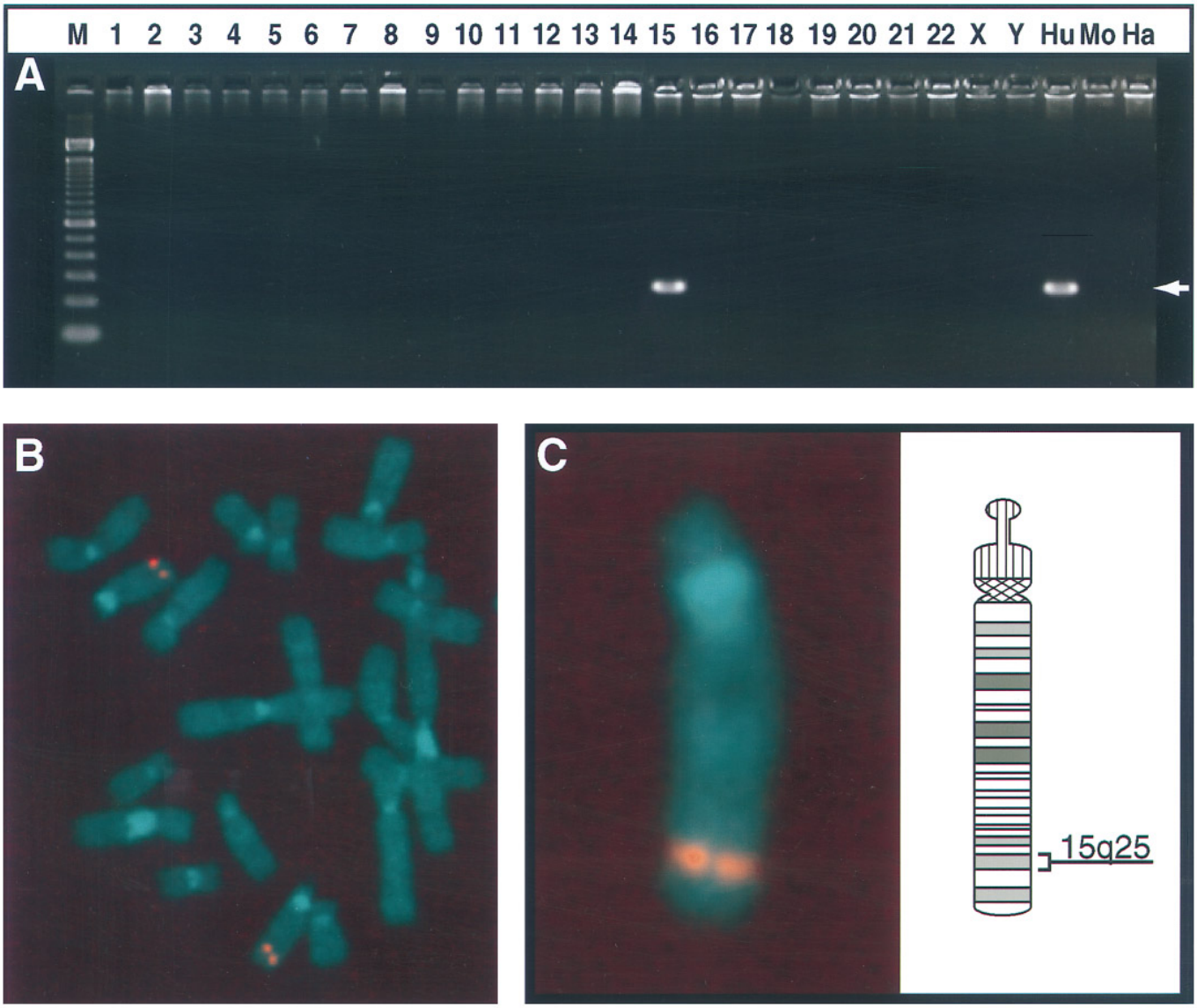

FIG. 1. Chromosomal localization of the human DNA polymerase gamma gene. (**A**) Oligonucleotide primers specific for *POLG* were used for PCR amplification of genomic DNA, and products were analyzed on an agarose gel stained with ethidium bromide. Lane **M,** 100-bp ladder; Lanes **1–22, X, Y,** somatic hybrid cell lines each containing a single human chromosome; Lane **Hu,** human; Lane **Mo,** mouse; Lane **Ha,** hamster. A 237-bp PCR product was detected in the lanes containing human chromosome 15 and human genomic DNA. (**B**) Partial normal lymphocyte metaphase demonstrating intense hybridization of *POLG* PAC clone 10K5 on the distal long arm of both copies of chromosome 15. (**C**) DAPI banding pattern and FLpter measurements are consistent with regional assignment of *POLG* to 15q25.

by free radical attack at the ribose ring and strand scission of mtDNA. These hypothetical nicked mtDNA molecules could then serve as the focal point for strand invasion and mispairing of mtDNA sequences, which in turn would form the substrate for either intramolecular recombination or aberrant mtDNA replication.

Little is known about the genes that control the sequence fidelity and structural integrity of the human mtDNA or their role in mitochondrial pathology. Highly oxidative tissues and tissues with high energy demands invariably have extraordinary high levels of mtDNA per cell; a ventricular cardiomyocyte can have upward of 50,000 copies of the mitochondrial genome. Due to its direct role in establishing mtDNA copy number and the maintenance of mtDNA sequence fidelity, it is of interest to map the nuclear gene that encodes the human mtDNA polymerase gamma (*POLG*).

Initial chromosomal assignment of the *POLG* gene was determined by polymerase chain reaction (PCR) analysis of the NIGMS human/rodent somatic cell hybrid mapping panel 2 (Coriell Institute for Medical Research, Camden, NJ). Oligonucleotide primers (hmtDNApolF, 5′-AAGGCCAGCCATTTTTCAGTAGCA-3′ and hmtDNApolR, 5′-GCATGGGGGACAGAACAAAGAACC-3′), derived from the *POLG* cDNA sequence (GenBank Accession No. X98093), were used in a PCR analysis of the hybrid mapping panel DNAs. On agarose gel electrophoresis, the predicted PCR product was observed only in the hybrid cell line DNA containing human chromosome 15 (Fig. 1A).

The 237-bp PCR product amplified from human genomic DNA with primers hmtDNApolF and hmtDNApolR was used as a probe to identify clones containing *POLG* in a 3× coverage PAC library (2). The PCR product was gel purified on low-melting-point agarose, labeled with [^{32}P]dCTP by random priming according to the manufacturer's instructions (Boehringer Mannheim), hybridized to the high-density PAC filters overnight at 42°C, washed to a stringency of 2× SSC, 0.1% (w/v) SDS at 55°C, and exposed to X-OMAT AR film for 6 days. PAC clones with plate addresses 10K5 and 10O5 showed a strong hybridization signal. DNA from both PAC clones was confirmed as positive for *POLG* by PCR.

Fluorescence *in situ* hybridization was used to refine the localization of *POLG* further. PAC clone 10K5 DNA was labeled with SpectrumOrange–dUTP (Vysis) by nick-translation (Gibco/BRL). The labeled DNA was purified over a Bio-Spin 6 column (Bio-Rad) and coprecipitated with 10 μg human Cot-1 DNA (Gibco/BRL). Hybridization was performed as previously described (4). Briefly, labeled PAC DNA (500 ng) was added to a 10-μl hybridization mix that contained 50% formamide, 2× SSC (pH 6.3), 10% dextran sulfate, denatured at 74°C for 2 min, and applied to normal human peripheral blood lymphocyte metaphase spreads for overnight hybridization at 37°C. The slides were then washed three times in 50% formamide, 2× SSC for 20 min each at 45°C. The hybridization signal was detected by two layers of FITC-conjugated avidin (Vector) and amplified with one layer of anti-avidin antibody (Vector). Slides were counterstained with 1 mg/ml DAPI (Boehringer Mannheim) in an antifade solution. Consistent with the results of somatic cell hybrid mapping, specific hybridization on both chromatids was detected on the distal long arm of chromosome 15 in all 42 metaphases examined (Fig. 1B). No hybridization was observed on any other chromosome. Hybridization signal fractional length from pter (FLpter) measurements were determined using the Fract Length-1 Probe Macro run in conjunction with NIH image 1.58 (Life Sciences Division, Los Alamos National Laboratory). Analysis of 26 examples of chromosome 15 yielded an average FLpter value of 0.864 (SD = 0.028). In agreement with the DAPI banding pattern, this value indicates a localization to 15q25 (Fig. 1C) (1).

No diseases with features of loss-of-energy syndromes have so far been mapped to 15q. However, the PAC clones described here will be useful in establishing the genomic structure of the mitochondrial DNA polymerase and determining the possible role of *POLG* mutations in the development of mtDNA mutations.

ACKNOWLEDGMENTS

We thank Daryl Leja and Nasser Parsa for their expert technical advice and assistance.

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Localization of the Human Transaldolase Gene (TALDO) to Chromosome 1p33–p34.1 by Fluorescence *in Situ* Hybridization and PCR Analysis of Somatic Cell Hybrids

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Received July 15, 1996; accepted December 6, 1996

Transaldolase catalyzes the transfer of a C3 fragment corresponding to dihydroxyacetone from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate, forming erythrose 4-phosphate and fructose 6-phosphate in the pentose phosphate pathway. The pathway provides mainly D-ribose 5-phosphate for nucleic acid synthesis and NADPH for lipid biosynthesis (9).

In human tissues, the transaldolase gene (TALDO) is expressed selectively in oligodendrocytes at high levels (2), possibly linked to production of large amounts of lipids as a major component of myelin. Multiple sclerosis (MS) lesions are characterized by a progressive loss of oligodendrocytes and demyelination of the central nervous system (8) and, moreover, production of autoantibody to transaldolase. The autoantibodies to transaldolase were detected in serum (29%) and cerebrospinal fluid (75%) of patients with MS (2). The cross-reactivity between HTLV-I gag protein and transaldolase revealed that the two proteins have a similar epitope and

Sequence data from this article have been deposited with the GenBank/EMBL Data Libraries under Accession No. U62747.

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GENOMICS **40,** 378–381 (1997)
ARTICLE NO. GE964561
0888-7543/97 $25.00