SHORT COMMUNICATION

Assignment of the Gene Encoding DNA Ligase I to Human Chromosome 19q13.2–13.3

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The gene encoding DNA ligase I has been mapped on human chromosome 19 by analysis of rodent–human somatic cell hybrids informative for this chromosome and by two-color fluorescence *in situ* hybridization. The DNA ligase I gene (*LIG1*) is localized to 19q13.2–13.3 and is distal to *ERCC1*, the most telomeric of three DNA repair genes on this chromosome. © 1992 Academic Press, Inc.

Human cDNAs encoding DNA ligase I, the major DNA ligase activity in proliferating cells, have been isolated by hybridization with oligonucleotides deduced from partial amino acid sequence of the purified bovine enzyme and by complementation of a *cdc9* temperature-sensitive DNA ligase mutant of *Saccharomyces cerevisiae* (Barnes *et al.*, 1990). Northern hybridization analysis with a human cDNA probe identified a 3.2-kb transcript. Southern hybridization between cloned cDNA sequences and *Eco*RI-digested genomic DNA identifies a discrete number of hybridizing bands (Fig. 1, lanes 6 and 13), indicating that the mRNA is encoded by a unique gene, *LIG1*.

The human chromosomal locus encoding DNA ligase I has been mapped by hybridization of a 1438-bp *Eco*RI cDNA fragment and a contiguous 1542-bp *Eco*RI fragment to genomic DNA from a panel of 13 rodent–human somatic cell hybrids of known karyotype (Barnes *et al.*, 1990). Human-specific hybridization signals correlated with the presence of human chromosome 19 and are shown for representative hybrids (Fig. 1). There is some cross-hybridization with the mouse genome, even at high stringency (Fig. 1, lanes 7 and 14), but human-specific hybridization is clearly discernible. The above two *Eco*RI restriction-fragment probes together span the entire coding region and 3′ untranslated region of the full-length cDNA. Size estimation and summation of genomic restriction fragments hybridizing to each probe (Fig. 1, lanes 6 and 13) predict a maximum size for the *LIG1* gene of about 50 kb.

The DNA ligase I cDNA probes were also used in hybridizations with *Eco*RI-digested genomic DNA from several somatic cell hybrids (Williams *et al.*, 1988) retaining fragments of human chromosome 19 (see Fig. 2). A positive signal was only seen with hybrid JDA16 (data not shown). The data obtained with these additional hybrids exclude the two terminal bands of this chromosome and are consistent with a regional map position for *LIG1* from 19p13.2 to 19q13.3.

The full-length human DNA ligase I cDNA was used to screen a 3× coverage of an arrayed human chromosome 19-specific cosmid library (de Jong *et al.*, 1989). Three positive cosmids were identified. Two of these cosmids have been analyzed and, along with an additional cosmid, form a contig. The overlap of these cosmids was detected using a high-density restriction enzyme fingerprinting strategy (Carrano *et al.*, 1989; Branscomb *et al.*, 1990). The span of the DNA ligase I positive cosmids in the contig is consistent with the size of the *LIG1* gene as estimated by Southern hybrid-

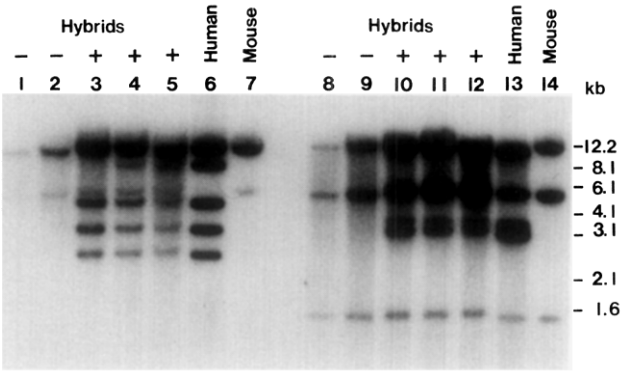

FIG. 1. Chromosomal localization of the *LIG1* gene. Southern blot of 10 μg *Eco*RI-digested human genomic DNA (lanes **6** and **13**), mouse DNA (lanes **7** and **14**), and DNA from a representative sample of a panel of rodent–human somatic cell hybrids (lanes **1** to **5** and **8** to **12**). Presence (+) or absence (−) of human chromosome 19 is indicated for each hybrid. Lanes **1** to **7** were probed with a 1438-bp *Eco*RI cDNA fragment; lanes **8** to **14** were probed with a contiguous 1542-bp *Eco*RI fragment. Hybridizations were carried out as described previously (Ref. (1)). Size markers (bacteriophage λ DNA restriction fragments) were run on the same gel and detected by hybridization with a ^{32}P-labeled λ probe.

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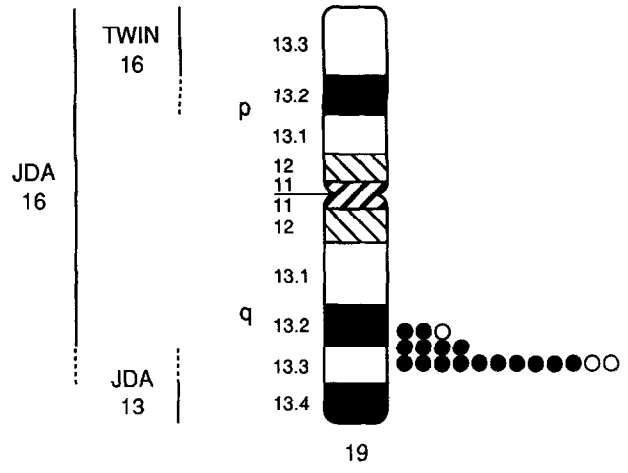

FIG. 2. Idiogram of G-banded human chromosome 19 showing *in situ* hybridization of a cosmid clone identified by a DNA ligase I cDNA probe. The fluorescent signal seen on both chromatids (●) or a single chromatid (○) was scored for 10 metaphase chromosome spreads of peripheral blood lymphocytes of a normal donor. No hybridization signals were seen on other chromosomes. The extent of chromosome 19 fragments present in rodent–human somatic cell hybrids, which were informative for DNA ligase I-specific probes, are shown by vertical lines. Broken lines indicate bands containing the breakpoint of the retained chromosome 19 fragment.

ization analysis of genomic DNA with DNA ligase I cDNA probes. Fluorescence *in situ* hybridization of one of the DNA ligase I-specific cosmids (f22135) to peripheral blood lymphocyte metaphase chromosomes banded with DAPI (4′, 6-diamidino-2-phenylindole) and actinomycin was carried out as described by Mohrenweiser *et al.* (1989) and Trask *et al.* (1991). As shown schematically in Fig. 2, the fluorescent signal localized entirely to bands 19q13.2 and 19q13.3. Assignment of the *LIG1* gene to this region by *in situ* hybridization is consistent with the broad localization determined by Southern hybridization analysis of rodent–human somatic cell hybrids retaining fragments of human chromosome 19 (see Fig. 2).

The human DNA repair genes *ERCC1* (van Duin *et al.*, 1986), *ERCC2* (Weber *et al.*, 1990), and *XRCC1* (Thompson *et al.*, 1990) have been mapped to the same region of human chromosome 19, *XRCC1* to q13.1–13.2, and *ERCC1* some 150 kb distal to *ERCC2* in q13.2–13.3 (Mohrenweiser *et al.*, 1989; Thompson *et al.*, 1989). *XRCC1* maps centromere proximal to *ERCC1* in somatic cell hybrid panels (Thompson *et al.*, 1989). The homologs of these three genes also constitute a highly conserved linkage group on Chinese hamster chromosome 9 (Thompson *et al.*, 1989). Two-color *in situ* hybridization experiments with metaphase chromosomes were performed as described elsewhere (Trask *et al.*, 1991) to position *LIG1* relative to *ERCC1*. Cosmids that were positive for a DNA ligase I cDNA probe (f22135) or *ERCC1* (f15123) were labeled with digoxigenin and biotin, respectively; their hybridization sites were labeled with fluoresceinated antibodies and Texas Red-conjugated avidin, respectively (Fig. 3). The green hybridization site of *LIG1* was telomeric of the red *ERCC1* hybridization site in 56% of 50 randomly selected chromatids analyzed. *LIG1* appeared centromeric of *ERCC1* in 14% of chromatids. The two probes were equidistant from the telomere in the remaining 30% of chromatids. These *in situ* hybridization data, together

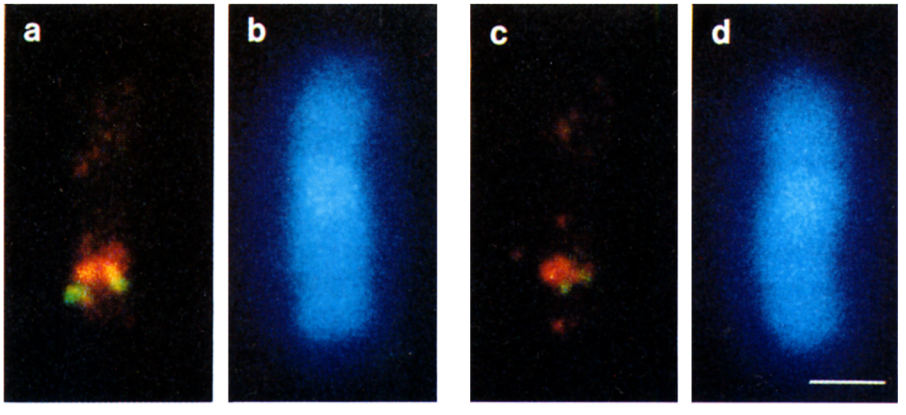

FIG. 3. Two-color fluorescence *in situ* hybridization of *ERCC1* (red) and *LIG1* (green) to chromosome 19 from a metaphase spread of a normal donor. The hybridization site of *LIG1* lies distal to that of *ERCC1* on three chromatids. The fourth chromatid is labeled with *LIG1* only. (**a** and **c**): *ERCC1* modified with biotin and labeled after hybridization with Texas Red; *LIG1* modified with digoxigenin and labeled after hybridization with FITC (green) (Ref. (8)). Repetitive sequences in the probes have not been completely suppressed by the addition of unlabeled genomic DNA and appear as a red/green speckling of the chromosomes. Red and green fluorescence in (**a**) and (**c**) were photographed simultaneously through a double band-pass filter (Omega, Brattleboro VT; excitation band passes centered around 490 and 560 nm, dichroic and emission band-pass filters centered around 530 and 650 nm). (**b** and **d**): DAPI fluorescence corresponding to panels (**a**) and (**c**), respectively. DAPI staining followed by incubation in actinomycin results in a low-resolution G-banding pattern along the chromosomes when viewed through a standard UV fluorescence filter set (Ref. (5)). Bar = 2 μm.

with previous genetic and physical data, indicate an order for the four genes on the long arm of chromosome 19 of

cen–*XRCC1–ERCC2–ERCC1–LIG1*–tel.

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