Localization of STCH to Human Chromosome 21q11.1

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STCH is a member of the stress70 chaperone family, which plays a major role in the processing of cytosolic and secretory proteins. Members of the stress70 protein chaperone family participate in protein processing events by binding denatured or misfolded peptide sequences and then releasing these polypeptide chains by an ATP-dependent mechanism. Members of this protein family possess an ATPase activity located in the highly conserved amino-terminal domain and a less well-conserved carboxy-terminal domain necessary for peptide binding (1, 3, 7).

The *Stch* gene encodes a 60-kDa peptide that is constitutively expressed in all human cell types and shares a high degree of amino acid identity with HSP70 and BiP (7). The protein differs from previously identified stress70 gene products by the presence of a unique hydrophobic signal sequence and the absence of a carboxy-terminal peptide-binding domain. This results in the protein being highly enriched in the lumen of a crude cellular microsome fraction and possessing an ATPase activity that, unlike other HSP70-like proteins, is not peptide inducible (5). Analysis of the rat *Stch* cDNA demonstrates that the unique properties of this C-terminal truncated HSP70-like molecule are conserved through mammalian evolution. As with the human *Stch* gene, the rat cDNA encodes a hydrophobic leader sequence, and the putative translation product lacks a C-terminal peptide-binding domain (Otterson and Kaye, in preparation).

To assign the *Stch* gene to a particular human chromosome, we mapped the gene by PCR analysis of a human/Chinese hamster somatic cell hybrid mapping panel (6). We developed PCR primers (primer 46, 5′-GCTTGTCTCAATCTGTGGATTTCC-3′; and primer 49, 5′-GATCGGATCCAGATTGCTTCAGTACTTATATAAACAG-3′) from the human cDNA sequence (5) that yield a 99-bp PCR amplification product from the human gene but not from its hamster homologue (Fig. 1A, lanes 2 and 3). This product is also seen in YAC 306F3 (lane 5) and in the hybrid containing chromosome 21 as its only human chromosome (lane 4) and is not seen in hybrids containing any of the other human chromosomes (data not shown).

Using a high-resolution chromosome 21 somatic cell hybrid mapping panel (4), *Stch* was localized to 21q11.1 between D21S215 and D21S373. As shown in Fig. 1B, *Stch* maps close

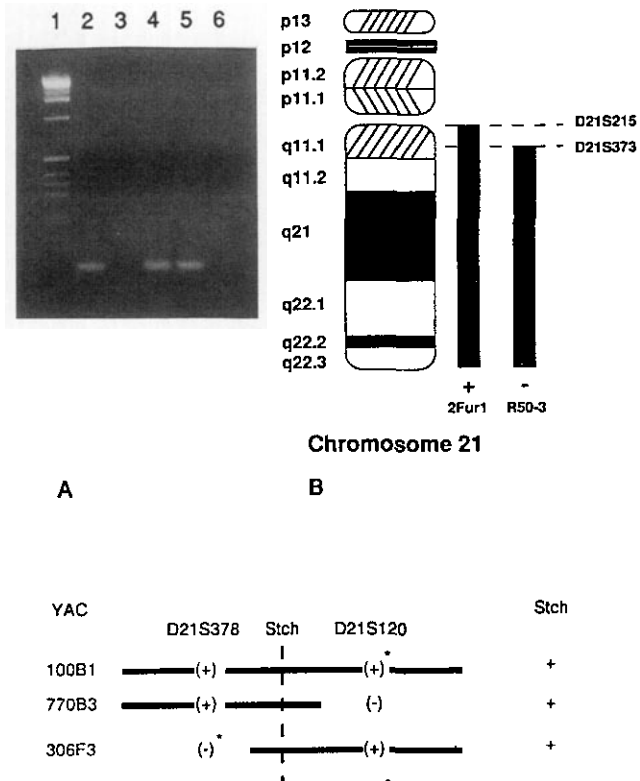

FIG. 1. Regional mapping of *Stch*. (**A**) PCR was performed using primers 46 and 49. Initial denaturation was at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s and 60°C for 30 s, with a final incubation at 72°C for 7 min. Samples were run on a 2% agarose gel and stained with ethidium bromide. (Lanes 1–6) DNA size markers, total human DNA (ER15L), CHO-K1 DNA, CHO hybrid containing human chromosome 21 DNA (R451-29C-5), YAC 306F3 DNA, and no template control. (**B**) Idiogram of G-banded chromosome 21 showing two hybrids, 2Fur1(+) and 6;21(−), from the high-resolution somatic cell hybrid mapping panel that regionally defines the location of *Stch*. (**C**) YAC *Stch* and STS analysis. STS markers that were not previously analyzed are indicated by asterisks. The putative position of the *Stch* gene is indicated by the vertical dashed line.

to the centromere of chromosome 21. Of the 23 radiation hybrid cell lines used in this experiment, only MRC2 gave results that were apparently inconsistent with this placement of the *Stch* gene. This cell line was found to contain sequence-tagged site (STS) markers D21S318, D21S286, D21S516, and D21S120, which are located on both sides of the proposed location for *Stch*. However, in repeated experiments MRC2 did not yield an amplification product with the *Stch* primers. This is most likely explained by the observations that the human DNA sequences in MRC2 are present as a circular molecule (4) and that deletions can result from the breakage of this molecule in different locations during cell growth and DNA isolation.

To refine further the mapping of *Stch*, eight YACs (124A7, 126B12, 676F7, 306F3, 100B1, 195D3, 770B3, and 599G6)

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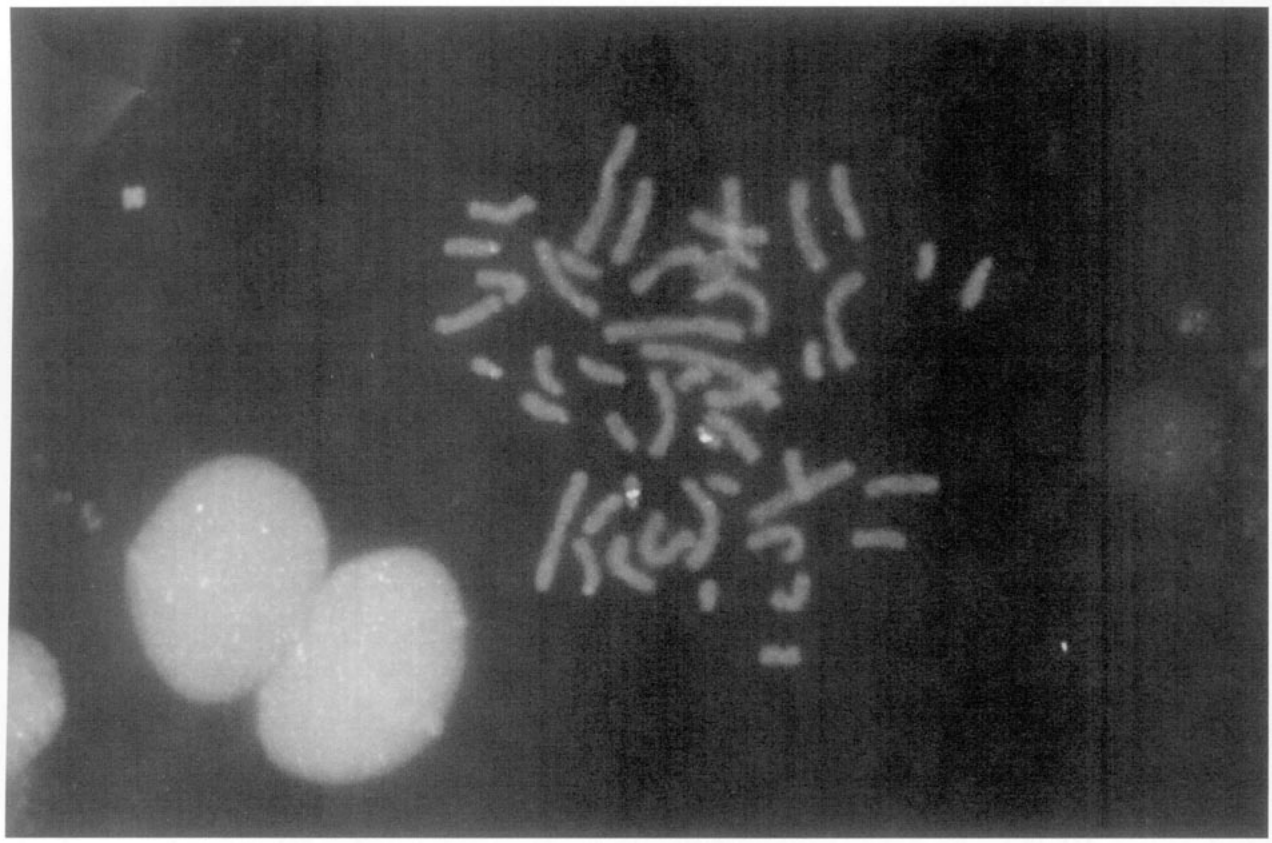

FIG. 2. Fluorescence *in situ* hybridization of human metaphase spread. Hybridization of YAC 306F3 results in a centromere-proximal signal on chromosome 21.

were identified by PCR screening of the 810 YAC set described by Chumakov *et al.* (2) as containing the gene and were colony purified. Consistent with the results from the hybrid mapping panel, all eight YACs map near the centromeric region of chromosome 21 (2). YAC 306F3 DNA was used as a probe for fluorescence *in situ* hybridization. This probe produced a signal located close to the centromere of chromosome 21 (Fig. 2), confirming the regional localization of *Stch*.

YACs both containing and lacking *Stch* were analyzed for the presence or absence of STS markers (Fig. 1C). YAC 306F3 does not contain D21S378. While YAC 100B1 was found to contain D21S120, YACs 254F7 and 770B3 were confirmed as lacking this marker. The observations that 770B3 contains *Stch* and D21S378 but lacks D21S120, that 254F7 contains D21S378 but lacks *Stch* and D21S120, and that 306F3 contains *Stch* and D21S120 but lacks D21S378 indicate that the *Stch* gene lies between D21S378 and D21S120. As such, *Stch* is the most centromere-proximal transcribed gene identified to date on chromosome 21.

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