the full-length cDNA described by Kaufman *et al.* (3). The larger mRNA species may represent a related transcript, which would be consistent with the genomic Southern blot results, or might be an alternative form of the 1.6-kb mRNA.

The p60 subunit of CAF-I is localized to 21q22.2, a region that has been correlated with many features of DS when triplicated (2, 4). As a subunit of a multimeric protein, the overexpression of p60 has potentially important implications for DS. For example, excess p60 could disrupt the stoichiometry of CAF-I, which could in turn affect the transcriptional availability of the DNA that CAF-I normally assembles into nucleosomes. This would suggest an indirect role for p60 in DS through the transcriptional regulation of many genes located elsewhere in the genome. Only one other gene involved in the regulation of gene expression, the human homologue of the *Drosophila* single-minded (sim) gene, has been described in this region (1). The significance of p60 is in its potential for affecting the transcriptional regulation of many genes not localized on chromosome 21. As the effects of DS occur in many tissues and to variable degrees between affected individuals, the overexpression of p60 makes it a potential candidate for substantial involvement in many of the phenotypes of DS. The ubiquitous expression pattern of p60 is consistent with an ability to affect the transcription of many genes in many tissues. The effect of such overexpression can now begin to be assessed in model systems *in vitro* as well as *in vivo.*

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Assignment of a New TGF-β Superfamily Member, Human Cartilage-Derived Morphogenetic Protein-1, to Chromosome 20q11.2

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The transforming growth factor-β (TGF-β) superfamily comprises a large group of signaling molecules that regulate cell growth, differentiation, and extracellular matrix formation through binding to specific transmembrane serine/threonine kinase receptors (1). We recently reported the isolation and characterization of two new members of the TGF-β family, designated cartilage-derived morphogenetic proteins-1 and -2 (CDMP-1, CDMP-2) (2). Northern analysis showed that both genes are predominantly expressed in cartilaginous tissues. *In situ* hybridization and immunostaining of sections from human embryos showed that CDMP-1 was predominantly found in the developing limb bud and throughout the cartilaginous cores of the developing long bones (2). *Cdmp1* was linked to markers on mouse chromosome 2 just proximal to *Src* (2). The closest linkage was observed with *Psp, Emv15,* and Growth differentiation factor-5 (*Gdf5*) in mice (8). This map location suggested close proximity to the brachypodism (*bp*) locus. Here, we have performed mapping and linkage analysis to define the locus of this gene within the human genome.

The human and rodent parental cell fusion procedure and the isolation and characterization of hybrids have been described previously (6, 7). The 41 human–hamster hybrids consisted of 29 primary hybrids and 12 subclones, and the 51 human–mouse hybrids consisted of 20 primary hybrids and 31 subclones. DNA (10 μg) was digested with *Eco*RI, separated by 0.7% agarose gel electrophoresis, transferred to nylon membrane, and hybridized with the 2.4-kb *Cdmp1* full-length cDNA probes at high stringency. The *Cdmp1* gene was detected as 2.6- and 5.9-kb bands that were readily distinguished from the 2.8- and 4.0- or 8.0-kb cross-hybridizing hamster or mouse bands, respectively. The *Cdmp1* gene was present in all 44 hybrids that retained chromosome 20 and

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TABLE 1

Two-Point Lod Scores between *Cdmp1* and Other Chromosome 20 Loci

	RFLP		PCR		
Locus	θ_{max}	Z_{max}	θ_{max}	Z_{max}	Region
D20S111	0.020	12.3	0.027	25.5	20q11.2 (3)
D20S191	0.000	12.6	0.000	20.2	20q11.2 (3)
D20S195	0.000	12.0	0.000	16.3	20q11.2 (3)
D20S174	[a]	[a]	0.043	19.0	20q11.2 (3)
D20S107	0.036	11.9	0.034	25.3	20q11.2 (3)

Note. Most likely recombination fraction (θ_{max}) and lod scores (Z_{max}) between *Cdmp1* and other loci. Values of $\geqslant 3$ for Z_{max} are considered significant evidence for linkage. All lod scores in this table are highly significant.

[a] There were no families segregating using the RFLP for D20S174.

absent in all 48 hybrids that lacked the chromosome. By contrast, *Cdmp1* segregated discordantly ($\geqslant$17%) with all other chromosomes.

The same cDNA probe was also used in Southern analysis of restriction enzyme-digested genomic DNA from 10 unrelated individuals. A diallelic restriction fragment length polymorphism (RFLP) was detected with *Hin*dIII, which gave two alleles at 7.2 (A1), 4.1, and 3.1 (A2) kb. The allele frequencies in the parents of the 40 CEPH families were 0.92 for A1 and 0.08 for A2. Linkage analysis of the 10 CEPH families that were segregating the RFLP revealed close linkage to chromosome 20 markers (Table 1).

More detailed linkage analysis utilized a $[CA]_n$ repeat in the 5′-untranslated region of *Cdmp1* (4). The oligonucleotide primer set flanking the $[CA]_n$ repeat was as follows: sense, 5′ TTCCAATTCC TGAGTTCAGG 3′; antisense, 5′ GAAGGAAGAA TGGCGTAATG 3′. The PCR products were then denatured, and an aliquot (4 μl) was applied to a 6% polyacrylamide gel in 1× TBE buffer, pH 8.0. Electrophoreses were performed at constant power (65 W) at room temperature for 3 h. Gels were dried, and band locations were determined by autoradiography. Thirty-five of the 40 CEPH families were informative for the $[CA]_n$ repeat, which comprised at least 10 alleles ranging from 164 to 182 bp by 2-bp difference. The allele frequencies in the CEPH parents were 0.019 for 182 bp, 0.165 for 180 bp, 0.139 for 178 bp, 0.177 for 176 bp, 0.044 for 174 bp, 0.038 for 172 bp, 0.089 for 170 bp, 0.032 for 168 bp, and 0.297 for 166 bp. There was no recombination observed between the $[CA]_n$ repeat polymorphism and the RFLP marker [$\theta = 0.000$, $Z_{max} = 21.98$]. The RFLP and dinucleotide repeat data were analyzed versus all relevant published loci in CEPH database Version 5 by two-point linkage analyses using LINKAGE 5.10 (5). Two-point linkage analysis with all relevant loci in the region shows close linkage of *Cdmp1* to all these loci, which span a total distance of 7 cM, with highly significant lod scores (Table 1). The *Cdmp1* gene is most closely linked to D20S191 and D20S195, which have been colocalized in the same physical contig in the 20q11.2 region (3). No recombinants were found between the *Cdmp1* marker and these two closely linked markers, but two and four recombinants were found with D20S111 and D20S174, respectively. These experiments show that the *Cdmp1* gene is located on chromosome 20q11.2, in a region on mouse chromosome 2 syntactic with the locus described for *Gdf5* (8).

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Localization of Staf50, a Member of the Ring Finger Family, to 11p15 by Fluorescence *in Situ* Hybridization

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The interferons (IFNs) are a family of secreted multifunctional proteins. First characterized for their antiviral func-

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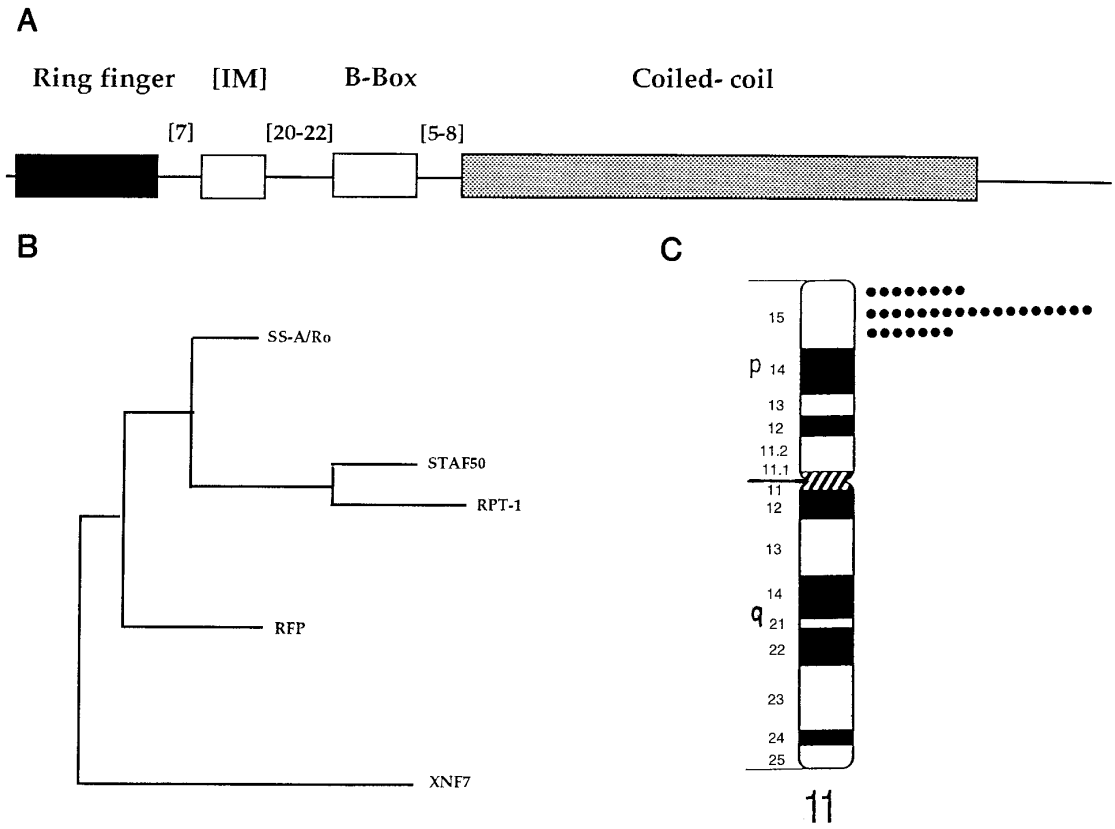

FIG. 1. (A) Diagrammatic representation of the [IM] B-box family. The solid box represents the Ring finger, the empty boxes represent the [IM] and B-box motifs, and the gray box represents the coiled–coil. The spaces between the motifs are shown in brackets. (B) Evolutionary tree performed using the Treealign software. (C) Idiogram of chromosome 11 showing the distribution of fluorescent spots on 11p15 for the 19 most resolved metaphases.

tions, it has now been established that they are involved in the control of cell proliferation and differentiation as well as in the regulation of the immune system (10). The transcriptional activation of specific genes in their target cells is an essential part of the cellular responses to IFNs. Although several IFN-regulated genes have been identified, the molecular mechanisms of IFNs remain poorly understood.

To search for as yet unidentified IFN-regulated genes, we have performed a differential screening on a cDNA library prepared from IFN-treated human lymphoblastoid Daudi cells. In the course of these studies we have isolated a human cDNA encoding a new member of the Ring finger superfamily, which we have referred to as Staf50 for stimulated transacting factor of 50 kDa (13). We have shown that Staf50 is induced by both human α/β IFN and human γ IFN in various human cell lines. The ability of Staf50 to down-regulate the transcription directed by the LTR promoter region of HIV-1 suggested that it might be involved in the antiviral process of IFNs against retroviral infections.

The comparison of the amino acid sequence of Staf50 with the sequences contained in the EMBL and GenBank databases revealed that the Staf50 protein shares homologies with three other members of the Ring superfamily: 44% homology with the mouse Rpt-1 protein (8), 40.5% with the human 52-kDa SS-A/Ro autoantigen (1), and 36% with the human Ret finger protein (11). The mouse Rpt-1 gene is selectively expressed in resting helper/inducer T cells and was shown to down-regulate gene expression directed by the HIV-1 promoter (8). The human 52-kDa SS-A/Ro is a component of the SS-A/Ro ribonucleoparticle recognized by autoantibodies in patients with systemic lupus erythematosus (1). The nuclear matrix-associated Ret protein has a transformation potential when fused with a tyrosine kinase domain (11).

Based on the nucleotide sequence, the cDNA structure, and the specificity of tissue expression, Staf50 can probably be identified as the human homolog of the mouse Rpt-1 gene (13). Multiple sequence alignment with the Clustralw software (6) was then used to determine the structural relationship between these different proteins. The analysis of their amino-terminal sequence alignment revealed that Staf50 is a member of the previously described Ring B-box subfamily (4). This subfamily is characterized by two highly conserved cysteine-rich motifs (the Ring finger and the B-box), with a predicted coiled–coil domain C terminal to the cysteine-rich motif (see diagrammatic representation in Fig. 1A). In addition, we have identified between the Ring finger and the B-box a new conserved basic motif that we have termed [IM] and that defines the Ring [IM] B-box subclass (Fig. 1A) as part of the Ring B-box subfamily. This subclass includes another Ring finger protein, the *Xenopus* nuclear factor 7 (XNF7) (9). The complete sequence alignment of these proteins including XNF7 was processed to elaborate an evolutionary tree using the Treealign software (5) available on the CITI-2/Bisance network (2). In keeping with the previously described phylogenetic relationship between Rpt-1 and the 52-kDa SS-A/Ro proteins (14), the phylogenetic tree presented in Fig. 1B suggests that Staf50 and 52-kDa SS-A/Ro have probably evolved from a common ancestor.