Locations of Human and Mouse Genes Encoding the *RFX1* and *RFX2* Transcription Factor Proteins

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RFX transcription factors constitute a highly conserved family of site-specific DNA binding proteins involved in the expression of a variety of cellular and viral genes, including major histocompatibility complex class II genes and genes in human hepatitis B virus. Five members of the RFX gene family have been isolated from human and mouse, and all share a highly characteristic DNA binding domain that is distinct from other known DNA binding motifs. The human *RFX1* and *RFX2* genes have been assigned by *in situ* hybridization to chromosome 19p13.1 and 19p13.3, respectively. In this paper, we present data that localize *RFX1* and *RFX2* precisely within the detailed physical map of human chromosome 19 and genetic data that assign *Rfx1* and *Rfx2* to homologous regions of mouse chromosomes 8 and 17, respectively. These data define the established relationships between these homologous mouse and human regions in further detail and provide new tools for linking cloned genes to phenotypes in both species.

RFX proteins constitute a growing family of site-specific DNA binding proteins that has been highly conserved throughout evolution. All members of the RFX family share a highly conserved and characteristic DNA binding domain that is distinct from all other known DNA binding motifs. RFX proteins have been implicated in a very diverse spectrum of biological systems, including regulation of the mitotic cell cycle in fission yeast (26), control of the immune response in mammals (21), and the expression of viral genes (19). Five different RFX proteins, RFX1 to RFX5, were recently identified in human and mouse (17, 21). Given the important functional roles assigned to this family of proteins, the locations of the various RFX genes are of special interest. *RFX1* and *RFX2* have been mapped by *in situ* hybridization to 19p13.1 and 19p13.2–p13.3, respectively (16). To provide new clues to the possible functions of both genes, we were interested in locating *RFX1* and *RFX2* more precisely within the chromosome 19p physical map and in mapping orthologous *Rfx1* and *Rfx2* genes in mouse.

To determine the positions of *RFX1* and *RFX2* in the human physical map, we used gene-specific probes to hybridize arrayed chromosome 19-specific cosmid libraries, as previously described (10). Cosmids constituting these libraries have been analyzed using a fingerprinting strategy and assembled into contigs (3, 4). Contigs were ordered and genomic distances between them estimated using two-color, three-cosmid analysis (2). The resulting maps of human chromosome 19 regions containing *RFX1* and *RFX2* are shown in Fig. 1. Our data place *RFX1* near the telomeric end of 19p13.1 between oncogenes *MEL* and *LYL1*, located approximately 1600 and 750 kb from those genes, respectively. *JUNB* lies just distal of *LYL1* and is thus positioned slightly less than 1 Mb from the *RFX1* gene in the telomeric direction. A fourth oncogene, *JUND*, is located an additional 1400 kb centromeric of *MEL* (Ref. 2; Fig. 1A). The human *RFX2* gene is located on 19p13.3 between the fucosyltransferase gene, *FUT5*, and the oncogene, *MLLT1* (Fig. 1B). These mapping data place *RFX2* within a 340-kb cosmid contig that also contains the *FUT3*, *FUT5*, and *FUT6* genes (Ref. 2; and data not shown). *VAV* and *C3* are located near each other on the centromeric side of *MLLT1* and are thus separated from *RFX2* by approximately 650 and 800 kb, respectively.

To locate *Rfx1* and *Rfx2* genes in the mouse, we followed the segregation of sequences corresponding to the two genes in 119 *Mus musculus* × *Mus spretus* interspecific backcross (IB) progeny (23, 24). The results of these experiments are summarized in Fig. 2. Similar to its human counterpart, *Rfx1* is linked to mouse homologs of *JUNB* and *JUND*, which are located in central mouse chromosome 8 (Mmu8; Ref. 6). As in human, mouse *Rfx1* is located nearest *Junb*, while *Jund* maps to the opposite end of the homology region (Fig. 2A). IB analysis also confirmed the very close linkage of *Rfx2*, *C3*, and *Vav* in distal Mmu17

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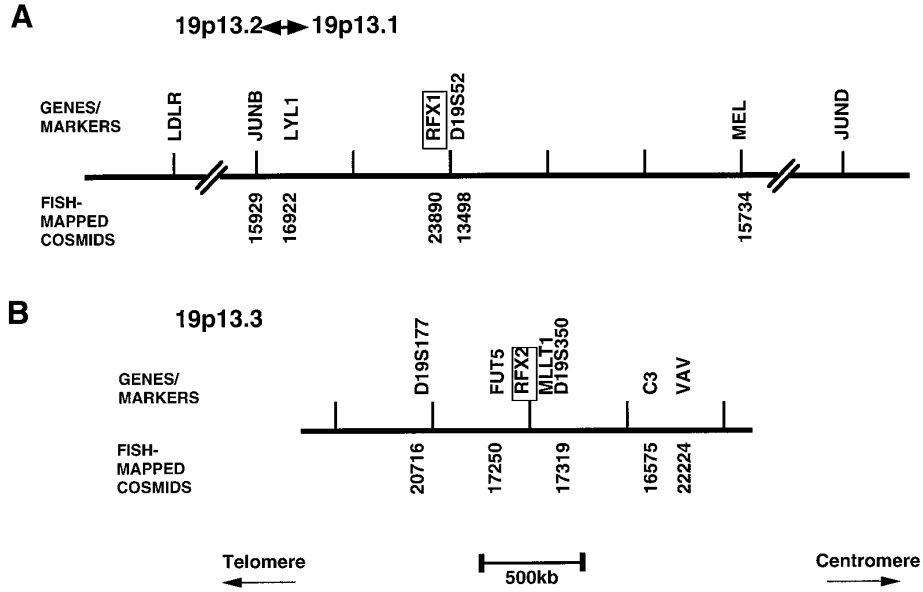

FIG. 1. Position of RFX genes on the detailed physical map of human chromosome 19 relative to the nearest polymorphic markers and genes of interest. (A) Region of map from 19p13.1 containing the *RFX1* gene. The gene *JUND*, which is shown separated from the rest of the loci by diagonal lines, is located about 1.5 Mb centromeric of *MEL*. Human *RFX1* and *RFX2* probes used in these studies have been described in a previous report (17). Other markers and mapping data have also been described (1, 2). (B) Region of map from 19p13.3 containing the *RFX2* gene. Distance measures between genes are provided by FISH measurements between cosmids that contain the gene or marker. The complete physical map of human chromosome 19 may be viewed via the Internet (URL: http://www-bio.llnl.gov/bbrp/genome/genome.html).

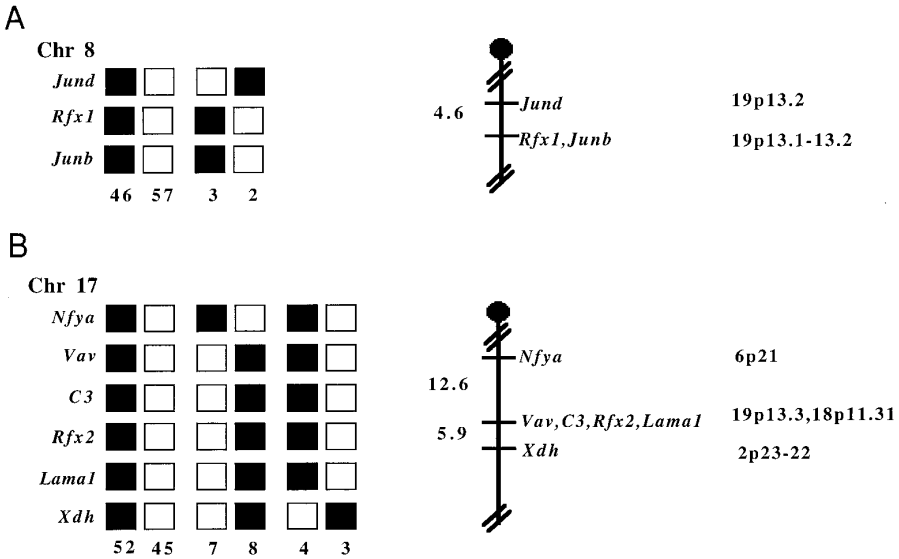

FIG. 2. Positions of the *Rfx1* and *Rfx2* loci on mouse chromosomes 8 and 17, respectively. Probes and variant restriction fragments used to follow the segregation of each gene in the 119 IB progeny [(C3Hf/Rl-*Mgf*^{Sl2ENURg}/+ × *M. spretus*) × C3Hf/Rl; Refs. 23, 24] were as follows: segregation of *Rfx1* (17) was traced with variant *Taq*I fragments [*M. musculus* (M) = 2.8 kb; *M. spretus* (S) = 4.7 kb]; *Rfx2* (17) with *Taq*I (M = 4.3 kb, S = 6.7 kb); *Jund* (18) with *Eco*RI (M = 23.0 kb, S = 16.2 kb); *Nfya* (25) with *Bam*HI (M = 5.4 kb, S = 9.4 kb); *C3* [7; obtained from the American Type Culture Collection (ATCC)] with *Hin*cII (M = 20 kb, S = 5.8 kb); *Vav* (11; from ATCC) with *Taq*I (M = 5.8 kb, S = 6.9 kb); and *Lama1* (15; from ATCC) with *Hin*cII (M = 16.2 kb, S = 6 kb). The *Xdh* probe, generated by PCR using primers derived from published sequence (5) (5′ CACCAGAAAACTGTAAATCC 3′ and 5′ ACACACACACACACACTATTC 3′) at an annealing temperature of 55°C, was mapped in *Bam*HI digests (M = 6.3 kb, S = 5.2 kb). Probes and variant fragments used to map *Junb* have been described (8). Markers were radioactively labeled and hybridized to Southern blots as described (22). Map positions were assigned using standard statistical methods (20) with the Map Manager data analysis program (13). (A) Assignment of *Rfx1* to mouse chromosome 8. (Left) Summary of the segregation patterns of *Rfx1* and flanking genes in 108 IB animals. Each column represents the chromosome inherited by groups of IB progeny from their C3Hf × *M. spretus* F_1 parent, with black boxes representing C3Hf alleles and white boxes denoting *M. spretus* alleles for a given locus. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. (Right) A partial chromosome 8 linkage map showing the location of *Rfx1* in relation to linked genes. Recombination distances between loci (in centimorgans) are indicated to the left of the map, and human homology regions are shown at right. (B) *Rfx2* maps in the distal region of mouse chromosome 17. (Left) Segregation patterns of *Rfx2* and flanking genes are summarized for 119 IB progeny, as detailed above. (Right) A partial chromosome 17 linkage map showing the location of *Rfx2* in relation to linked genes.

(Fig. 2B). The three genes, which are genetically inseparable in this IB system, are located approximately 12.6 cM (±3.0 cM) distal of the nuclear factor gene *Nfya*, in good agreement with previously published data (9, 12). The mouse gene encoding laminin A (*Lama1*), whose human counterpart maps to 18p11.2–p11.3 (14), is also genetically inseparable from *Rfx2*, *C3*, and *Vav* in this IB system, indicating a more proximal position for this gene than has been previously reported (9). In good agreement with previously published results, these IB data placed the murine *Xdh* gene 5.9 cM (±2.4 cM) distal of *C3*, *Vav*, and *Rfx2* (Fig. 2B).

These assignments add new mouse genes to well-established intervals of mouse–human homology and further define the physical maps of human chromosomes 19p13.1–p13.2 and 19p13.3, respectively. Using the human chromosome 19 physical map as a guide (1), we can predict the approximate sizes of homology regions including the mouse *Rfx1* and *Rfx2* genes and gain some potentially useful clues regarding gene organization in each region. If these related human and mouse regions are as similar as genetic data suggest, the interval of Mmu8 that carries 19p13.1–p13.2 gene homologs should span approximately 8 Mb in total length. Analogies between the Mmu17 and the human chromosome 19p maps further invite the prediction that the murine segment containing *C3*, *Vav*, and *Rfx2* should be relatively compact, spanning at least 1 Mb but not more than 3 Mb in total length (1). Physical characterization of murine regions surrounding both the *Rfx1* and the *Rfx2* genes is now under way and should allow us to confirm these predictions directly in the near future. The well-developed physical maps of human chromosome 19p should prove to be a useful tool for researchers interested in the study of genes and phenotypes mapping within these related mouse genomic regions.

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