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A 6000 kb segment of chromosome 1 is conserved in human and mouse

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A murine linkage map generated from analyses of 428 meiotic events in an interspecific cross and pulsed field gel electrophoresis allowed examination of the genomic organization of a 6000 kb segment of mouse and human chromosome 1. Analysis of five genes within this syntenic segment of both species revealed striking conservation of gene order, intergenic distance and, to a lesser extent, CpG dinucleotides. In the mouse, meiotic crossover events were not evenly distributed; a hot spot for meiotic recombination was coincident with a CpG-island. These studies provide a practical approach to aid physical mapping of the human genome and a model for determining the molecular principles that govern meiotic recombination. In addition, these findings demonstrate profound conservation of genomic organization over mammalian evolution.

Key words: chromosome 1/'CpG-islands'/evolution/genomic organization/meiotic recombination

Introduction

Large segments of mammalian chromosomes appear to have been conserved during evolution. More than 50 autosomal regions have been identified, comprising >300 genes, which are syntenic in man and mouse (Searle *et al.*, 1987; Lalley *et al.*, 1988). Conserved chromosomal segments exemplify relics of ancestral linkage groups which either have not yet been disrupted by stochastic translocations and inversions, or are protected from such chromosomal rearrangements because of regulatory or functional interactions between loci (Nadeau and Taylor, 1984). Delineation of genomic organization within linkage groups conserved between mouse and man should engender a better understanding of molecular events underlying mammalian evolution, identification of gene families and development of murine models of human hereditary diseases. Until recently, however, determination of the extent of genomic conservation within mammalian syntenic groups was limited by imprecision of gene localization based largely on somatic cell hybridization, *in situ* hybridization to metaphase chromosome spreads or incomplete genetic maps. The advent of precise genetic and long-range physical maps should permit examination of these questions.

Previous studies have identified a large linkage group conserved between human chromosome (Chr.) 1q21-32 and distal mouse Chr. 1 (Seldin *et al.*, 1988a,b; Kingsmore *et al.*, 1989a). In comparative mapping studies using DNA from a large panel of interspecific backcross mice, 16 genes have been positioned within this syntenic group, which spans 30 centi-Morgans (cM) on mouse Chr. 1. Within error of gene localization, all appear to be arranged colinearly in human and mouse (with opposite orientation with respect to the centromere). The current study was undertaken to examine in detail genomic organization of a 6000 kb segment of this conserved linkage group. Using pulsed field gel electrophoresis (PFGE) we show that within this syntenic group, gene order, intergene distance and, to a lesser extent, distribution of CpG-islands are similar in human and mouse. Furthermore, comparison of physical and recombinational distances between these genes in the mouse provides evidence that meiotic crossovers are not uniformly distributed along mammalian autosomes.

Results

Interspecific recombinational map of distal mouse Chr. 1

The murine genes encoding the α3-subunit of Na,K-ATPase (*Atpa-3*), the α-subunit of the high-affinity Fc receptor for IgE (*Fce1a*), serum amyloid P-component (*Sap*), C-reactive protein (*Crp*), erythroid α-spectrin (*Spna-1*), and a family of interferon-induced genes (provisionally designated *Ifi202, Ifi203* and *Ifi204*) were mapped by linkage analysis of restriction fragment length polymorphisms (RFLPs) in genomic DNA samples generated from [(C3H/HeJ-*gld/gld* × *Mus spretus*)F1 × C3H/HeJ-*gld/gld*] backcross mice. RFLPs were determined by Southern blot hybridization of DNA from C3H-*gld/gld* parental mice and (C3H-*gld/gld* × *M.spretus*)F1 mice digested with various restriction endonucleases. *Mus spretus* was chosen as the second parent because of the relative ease of detection of informative RFLP in comparison with crosses using conventional inbred strains. Figure 1 shows unique RFLPs (*M.spretus*) present in the F1 mice for *Atpa-3, Fce1a, Sap, Crp, Ifi202/Ifi204* and

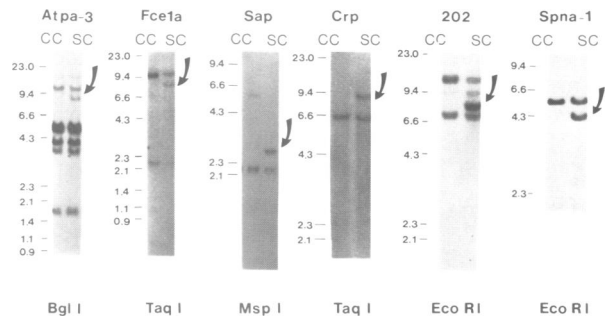

Fig. 1. Southern blot identification of unique *M.spretus* RFLPs detected with *Atpa-3, Fce1a, Sap, Crp, Ifi202* and *Spna-1* gene probes. Restriction endonucleases are indicated at the bottom and molecular size standards (in kb) are shown at the left of each panel. Arrows signify bands present in DNA from (C3H-*gld/gld* × *M. spretus*)F1 (SC) but not in C3H-*gld/gld* (CC) mice.

Table I. Gene mapping using C3H/HeJ-*gld/gld* × *M.spretus*)F1 × C3H/HeJ-*gld/gld* backcross mice

Murine genes	Number of recombination events[a]								Human homolog[b]	
	None		One							
Ly-17	CC[c]	SC	CC ×	SC ×	CC	SC	CC	SC	*CD32*	1q23-24
Atpa-3	CC	SC	SC	CC	CC	SC	CC	SC	*APT1A2*	1q
Fce1α	CC	SC	SC	CC	CC	SC	CC	SC	*FCE1A*	1q21-23
Sap	CC	SC	SC	CC	CC	SC	CC	SC	*APCS*	1q12-23
Crp	CC	SC	SC	CC	CC ×	SC ×	CC	SC	*CRP*	1q12-23
Ifi202/Ifi204	CC	SC	SC	CC	SC	CC	CC	SC	NI[d]	
Spna-1	CC	SC	SC	CC	SC	CC	CC ×	SC ×	*SPTA1*	1q22-25
D1Pas1	CC	SC	SC	CC	SC	CC	SC	CC	NI[e]	Xp11
No. of mice	329[f]	64	5	3	3	0	16	5		
	396		8		3		21			

[a]Columns indicate the genotype of individual backcross mice. Genotypes for mouse gene probes were determined by RFLPs illustrated in Figure 1 and Kingsmore *et al.* (1989a). With the gene order given, no double or multiple crossovers were seen.
[b]Designated nomenclature of human homologs and their chromosomal assignment based on *in situ* hybridization to metaphase chromosome spreads (Heubner *et al.*, 1985; Floyd-Smith *et al.*, 1986; Yang-Feng *et al.*, 1988; Tepler *et al.*, 1989).
[c]CC, C3H/HeJ homozygous genotype. SC, F1 genotype; ×, crossover.
[d]NI, human homologs of *Ifi202, Ifi203* or *Ifi204* not yet identified.
[e]NI, *in situ* hybridization of human chromosomes with *D1Pas1* detected predominantly human Chr. X (15.3% of silver grains, with 58% localized to Xp11) and showed a minor peak with human Chr. 6p (5.7% of silver grains) (M.G.Mattei and P.Leroy, unpublished results).
[f]The larger number of mice typing as C3H homozygous reflects selection of many of the backcross mice for the *gld/gld* phenotype consistent wiht a previous study mapping the *gld* gene on distal mouse Chr. 1 (Seldin *et al.*, 1988a).

Spna-1 gene probes. Segregation analysis was examined in 428 backcross mice typed with these RFLPs and also by previously described RFLPs detected with probes for *Ly-17* (equivalent to human *CD32*) and the testis-specific gene *D1Pas1* (previously referred to as *Pl-10*) (Seldin *et al.*, 1988a; Kingsmore *et al.*, 1989a). At each locus, backcross mice displayed either the homozygous C3H (CC) or the heterozygous F1 pattern (SC). Gene order was established by minimization of chromosome crossover events. The gene order given in Table I resulted in elimination of double crossovers. RFLPs associated with *Atpa-3, Fce1a, Sap* and *Crp* were tightly linked, with no recombinants evident in 428 meiotic events ($r = 0.0$ cM; $\underline{r} = 0.0$ cM, $\bar{r} = 0.9$ cM, 95% confidence limits for binomial distribution) and mapped 2.0 cM telomeric to *Ly-17* (*CD32*) on mouse Chr. 1 (Table I). *Spna-1* and *Ifi202/Ifi204* also co-segregated in 428 backcross mice, and map 0.7 cM telomeric to *Atpa-3, Fce1a, Sap* and *Crp* and 6.0 cM centromeric to *D1Pas1* on mouse Chr. 1 (Table I).

The chromosomal band location of human homologs of these genes, as determined by *in situ* hybridization, are indicated in Table I. With the exception of the human homolog of murine *D1Pas1*, which is not a member of the conserved linkage group, all map in the vicinity of human Chr. 1q21-23.

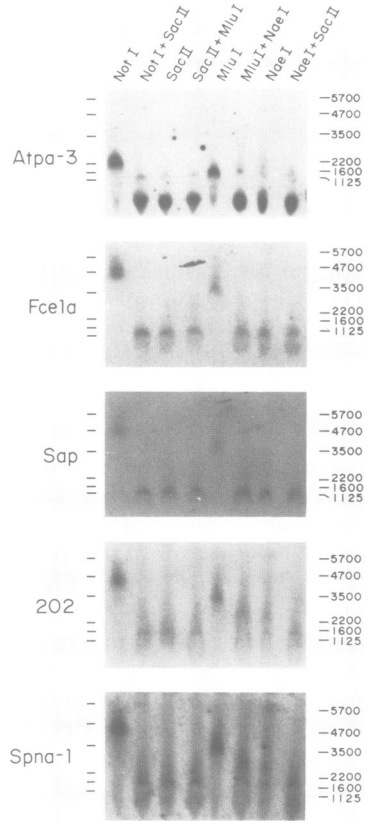

Fig. 2. Autoradiographs of a pulsed field gel Southern blot sequentially hybridized with gene probes *Atpa-3, Fce1a, Sap, Ifi202* and *Spna-1*. C3H/HeJ-*gld/gld* DNA was separated by pulsed field electrophoresis using ramped pulses from 15 to 90 min. Gene probes are indicated to the left of each panel. Restriction endonucleases are indicated at the top and molecular size standards in kb are shown to the right of each panel. Hybridization of this filter with probes *Ifi203* and *Ifi204* gave identical bands to *Ifi202*; hybridization of this filter with *Crp* gave identical bands to *Sap* (data not shown). *Fce1a, Sap, Crp, Ifi202, Ifi203, Ifi204* and *Spna-1* probes all detected 4500 kb *Not*I and 3500 kb *Mlu*I restriction fragments, indicating that *Fce1a, Sap, Crp, Ifi202, Ifi203, Ifi204* and *Spna-1* are located within 3500 kb. *Ifi202, Ifi203, Ifi204* and *Spna-1* recognized a common 1500 kb fragment with *Sac*II and common 1500 and 2300 kb *Nae*I bands. *Fce1a, Sap* and *Crp* hybridized to 1150 kb *Nae*I and *Sac*II fragments. *Atpa-3* gave disparate sized bands with all of these endonucleases. *Sac*II/*Not*I, *Sac*II/*Mlu*I, *Nae*I/*Not*I and *Nae*I/*Mlu*I double digests gave identical bands to *Sac*II or *Nae*I alone with all probes, indicating that the *Sac*II and *Nae*I sites are internal to *Not*I and *Mlu*I sites.

Physical map of a 6900 kb segment of distal mouse Chr. 1

In view of their genetic proximity, physical mapping studies of *Atpa-3, Fce1a, Sap, Crp, Ifi202, Ifi203, Ifi204* and *Spna-1* were undertaken using PFGE. High mol. wt genomic DNA samples from C3H-*gld/gld* lymphocytes were examined by Southern blot analysis after digestion with rare cutting restriction enzymes and PFGE. All DNA samples were derived from lymph nodes of C3H-*gld/gld* mice in order to avoid RFLPs due to differing tissue methylation patterns. Filters were hybridized sequentially with each of the eight probes.

Fce1a, Sap, Crp, Ifi202, Ifi203, Ifi204 and *Spna-1* probes all detected 4500 kb *Not*I, 3300 kb *Mlu*I and 1000 and 1800 kb *Sal*I restriction fragments (Figure 2, and data not shown). *Not*I/*Mlu*I and *Mlu*I/*Sal*I double digests gave

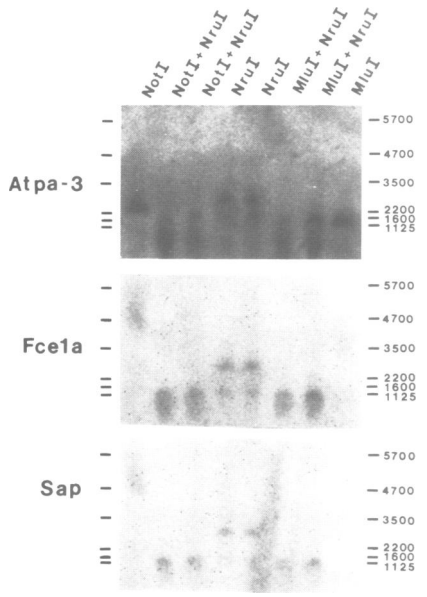

Fig. 3. Autoradiographs of a pulsed field gel Southern blot sequentially hybridized with gene probes *Atpa-3*, *Fce1a* and *Sap*. C3H/HeJ-*gld/gld* DNA was separated by pulsed field electrophoresis using ramped pulses from 15 to 90 min. Gene probes are indicated to the left of each panel. Restriction endonucleases are indicated at the top and molecular size standards in kb are shown to the right of each panel. *Atpa-3*, *Fce1a* and *Sap* probes all detected a 2800 kb *Nru*I band. *Fce1a* and *Sap* also detected a 1200 kb *Nru*I band, while *Atpa-3* also detected a 1600 kb *Nru*I band. Thus *Atpa-3*, *Fce1a* and *Sap* are all located within 2800 kb.

identical restriction fragments to *Mlu*I or *Sal*I single digestions respectively, indicating these *Sal*I cleavage sites to be internal to the *Mlu*I sites, which in turn were internal to the genomic *Not*I sites (Figure 3, and data not shown). Thus *Fce1a, Sap, Crp, Ifi202, Ifi203, Ifi204* and *Spna-1* were located within 1000 kb on mouse Chr. 1. *Atpa-3* hybridized to *Not*I, *Mlu*I and *Sal*I restriction fragments of different size to *Fce1a, Sap, Crp, Ifi202, Ifi203, Ifi204* and *Spna-1*. These data combined with the gene linkage results (Table I) indicate that *Atpa-3* is located centromeric to the 4500 kb *Not*I fragment (Figure 2). *Atpa-3* recognized 1600 and 2800 kb *Nru*I bands. *Fce1a* and *Sap* also identified a 2800 kb *Nru*I fragment, in addition to a 1200 kb band. *Not*I/*Nru*I and *Mlu*I/*Nru*I double digests gave a single 1200 kb band with *Fce1a* and *Sap*, and a 1600 kb band with *Atpa-3*, confirming that the 2800 kb *Nru*I band was common to *Atpa-3, Fce1a* and *Sap*, and contained an internal *Nru*I site which divided it into 1200 and 1600 kb segments (Figure 3). Since *Ifi202, Ifi203, Ifi204* and *Spna-1* were not located on this 2800 kb *Nru*I fragment, the minimum distance separating *Atpa-3* and the interferon-activated gene family was 1200 kb and the maximum distance 2800 kb (Figure 4a).

Whereas *Ifi202, Ifi203, Ifi204* and *Spna-1* recognized a common 1700 kb band with endonucleases *Sac*II, *Nae*I, *Bss*HII or *Nru*I, *Fce1a, Sap* and *Crp* did not, but instead hybridized to common 1000 kb *Sac*II and *Nae*I fragments (Figure 2). Double digests performed with *Sac*II, *Nae*I and *Nru*I showed cleavage sites for these enzymes to be coincident, indicating the presence of a CpG-island (Lindsay and Bird, 1987) separating *Ifi202, Ifi203, Ifi204* and *Spna-1* from *Fce1a, Sap* and *Crp* (Figures 2 and 4a). *Mlu*I/*Sac*II and *Mlu*I/*Nae*I double digests revealed that all of these *Sac*II and *Nae*I sites were internal to the 3300 kb *Mlu*I fragment (Figure 2). Previous studies have shown that *Ifi202, Ifi203* and *Ifi204* probes hybridize to a family of at least six sequence-related interferon-activated genes which are clustered within 450 kb on mouse Chr. 1 (Kingsmore *et al.*, 1989b). Furthermore, *Sap, Ifi202* and *Spna-1* were shown to be physically linked, in that order, between an interval of 450 and 1000 kb. In the present report, *Sap* hybridized to *Sac*II and *Nae*I bands of size 660 and 1000 kb, while *Fce1a* hybridized to 220 and 1000 kb *Sac*II and *Nae*I bands (data not shown). Since *Sap, Ifi 202, Ifi203, Ifi204* and *Spna-1* are contiguous genes, *Fce1a* must be centromeric to *Sap* (Figure 4a).

Using additional restriction endonucleases and multiple double and partial digests, a genomic restriction map of 6900 kb encompassing these genes was generated (Figure 4a). Further localization of *Crp*, however, was not possible due to poor cross-hybridization of this human cDNA probe on double-digest membranes. The gene order was determined to be: centromere–*Atpa-3*–*Fce1a*–*Sap*–*Ifi202/Ifi203/Ifi204*–*Spna-1*–telomere, where *Crp* is adjacent to *Sap* and *Fce1a*. The length of the genomic segment occupied by these genes is 2780–3220 kb.

Physical map of a 6100 kb segment of human Chr. 1q

Physical mapping studies of the human homologs of *Atpa-3* (*APT1A2*), *Fce1a* (*FCE1A*), *Sap* (*APCS*), *Crp* (*CRP*) and *Spna-1* (*SPTA1*) were undertaken by PFGE of high mol. wt DNA samples from human peripheral blood lymphocytes. *FCE1A, APCS, CRP* and *SPTA1* gene probes all hybridized to 2300 kb *Not*I, 3200 kb *Mlu*I, 2200 kb *Sal*I and 2300 kb *Nru*I fragments (Figure 5), indicating these genes to be located within 2200 kb. *APT1A2* recognized disparate fragments with these endonucleases (Figure 5). Partial *Nru*I digestion, however, gave additional bands of 3400 and 4300 kb, which were common to all probes, including *ATP1A2* (Figure 5). Thus *ATP1A2, FCEC1A, APCS, CRP* and *SPTA1* are all located within a 3400 kb segment of human Chr. 1 (Figure 4b).

The order of *ATP1A2, FCE1A, APCS, CRP* and *SPTA1* was determined with further restriction endonucleases and electrophoresis conditions which resolve 50–1200 kb DNA molecules (Figure 6). *Cla*I digestion gave disparate bands with each of the gene probes, demonstrating that they recognize unique DNA sequences (Figure 6). *FCE1A, APCS* and *CRP* recognized 550, 610, 780, 860 and 920 kb *Sal*I, and 650 kb *Nae*I fragments, whereas *SPTA1* and *ATP1A2* gave different sized bands, indicating that *FCE1A, APCS* and *CRP* are located within 550 kb (Figures 6 and 7). *APCS* and *CRP* probes detected further common bands with *Nae*I and *Nru*I enzymes, the shortest being a 290 kb *Nae*I fragment, placing *APCS* adjacent to and within 290 kb of *CRP* (data not shown). *FCE1A* and *APCS* recognized common 710 kb *Cla*I and 500 kb *Cla*I/*Nae*I fragments, placing *FCE1A* next to *APCS* (Figures 6 and 4b). Additional restriction endonucleases and informative double digests were used to generate a genomic restriction map of >6100 kb encompassing these genes (Figure 7). The gene order was determined to be: *ATP1A2–FCE1A–APCS–CRP–SPTA1*. The length of the genomic segment occupied by these genes is 2710–3400 kb.

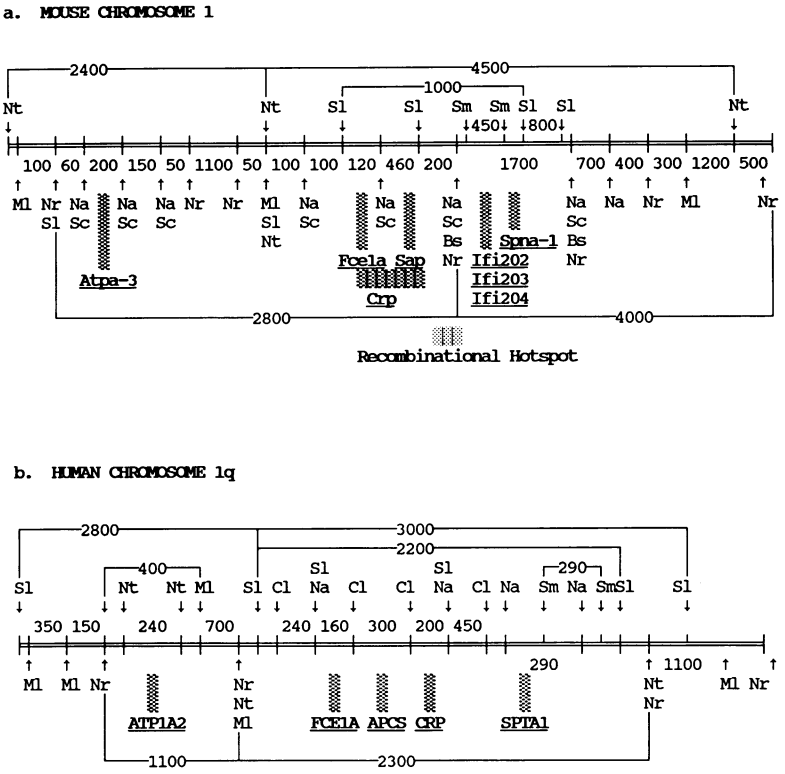

Fig. 4. Long-range restriction maps of a 6900 kb segment of distal mouse Chr. 1 (**a**) and of a 6100 kb segment of human Chr. 1q (**b**). *Not*I (Nt), *Mlu*I (Ml), *Sal*I (Sl), *Nru*I (Nr), *Nae*I (Na), *Sac*II (Sc), *Sma*I (Sm) and *Bss*HII (Bs) restriction endonuclease cleavage sites are indicated with arrows. Positions of the murine or human genes encoding the α3-subunit of Na^+K^+-ATPase (*Atpa-3* or *ATP1A2* respectively), the α-subunit of the Fc receptor for IgE (*Fce1a* or *FCE1A* respectively), serum amyloid P-component (*Sap* or *APCS* respectively), C-reactive protein (*Crp* or CRP respectively) and erythroid α-spectrin (*Spna-1* or *SPTA1* respectively) and of a family of murine interferon-activated genes (provisionally designated *Ifi202, Ifi203* and *Ifi204*) are shown with cross-hatched blocks. Restriction fragment lengths are given in kb. A hotspot for meiotic recombination between *Sap* and *Ifi202/Ifi204* (which contained all three crossovers observed in 428 meioses in this region) is indicated by a cross-hatched block (**a**). The maximum distance between *Sap* and *Ifi202/Ifi204* was determined as follows: *Fce1a, Sap, Ifi202, Ifi203, Ifi204* and *Spna-1* were all located, in that order, on a 1000 kb *Sal*I fragment. *Spna-1* was located within 460−590 kb of the telomeric end of this *Sal*I fragment, while the interferon-induced gene family occupy ~170 kb proximal to this 460 kb fragment (Kingsmore *et al.*, 1989b). *Sal*I/*Nae*I double digest revealed that *Fce1a* was located within 120 kb of the centromeric end of the 1000 kb *Sal*I fragment (data not shown). Thus the maximal distance separating *Sap* and *Ifi202/Ifi204* is 1000 − (460 + 170 + 120) = 250 kb.

Comparison of physical maps of a syntenic region of mouse and human Chr. 1

Comparison of genomic restriction maps of human and mouse Chr. 1 revealed a marked conservation of genomic organization (Figure 4a and b). The order of the four genes unambiguously mapped by PFGE in both species was the same: *ATP1A2/Atpa-3*−*FCE1A/Fce1a*−*APCS/Sap*−*SPTA1/Spna-1*. *Crp* was not definitively mapped in the mouse using PFGE due to inadequate cross-hybridization of the human cDNA probe. However, localization of *Crp* as determined by genetic assignment and partial physical mapping was consistent with the location of its human homolog on human Chr. 1 (Table I; Figure 4a and b). Lengths of genomic segments separating each of the genes were calculated from physical mapping data for both species (Table IIa). Comparison of intergene distances revealed striking similarity in mouse and human. Distances separating all gene combinations examined were, within the limits of the physical mapping performed, the same in mouse and human (Table IIa).

Comparison of the molecular sizes of the major band(s) observed upon hybridization of gene probes to DNA digested with a variety of infrequently cutting restriction endonucleases, which have recognition sequences rich in unmethylated CpG dinucleotides, revealed many similarities in mouse and human (Table IIb). Also similar in both species were the unusually large *Not*I and *Mlu*I fragments with *FCE1A/Fce1a, CRP/Crp, APCS/Sap* and *SPTA1/Spna-1* gene probes (Table IIb). Average *Not*I or *Mlu*I fragment sizes for human and mouse genomic DNA are ~1000 kb (Smith *et al.*, 1986).

While some differences in restriction fragment lengths were observed, the resultant genomic restriction maps were similar (Figure 4a and b). Thus, *Atpa-3* and its human homolog were physically linked to *Fce1a* or *FCE1A* respectively on the basis of a common *Nru*I fragment of similar size in both species. All other restriction endonucleases had cleavage sites between these genes.

Comparison of physical and genetic maps of distal mouse Chr. 1

The order of *Atpa-3, Fce1a, Sap, Crp, Ifi202/Ifi203/Ifi204* and *Spna-1* on distal mouse Chr. 1 as determined by minimization of chromosome crossover events (Table I) was in agreement with the order based upon physical linkage studies (Figure 4a). PFGE was useful in determining the order of genes which were tightly linked on a genetic map.

Comparison of physical and genetic distances among genes localized to this autosomal segment revealed striking disparities. While *Atpa-3* and *Sap* were physically separated

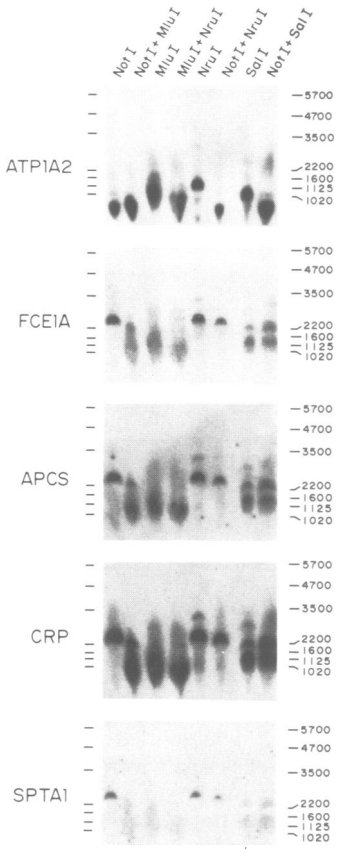

Fig. 5. Autoradiographs of a human pulsed field gel Southern blot sequentially hybridized with gene probes *ATP1A2, FCE1A, CRP, APCS* and *SPTA1*. Human PBL DNA was separated by pulsed field electrophoresis using ramped pulses from 15 to 90 min. Gene probes are indicated to the left and restriction endonucleases above each panel. Molecular size standards in kb are to the right of each panel. *ATP1A2, FEC1A, CRP, APCS* and *SPTA1* probes all detected a 3400 kb *Nru*I band, indicating that all of these genes are located within 3400 kb. *FCE1A, CRP, APCS* and *SPTA1* probes all detected 2300 kb *Not*I, 2200 and 2900 kb *Sal*I and 2300 kb *Nru*I restriction fragments, indicating *FCE1A, CRP, APCS* and *SPTA1* are located within 2200 kb. In **lanes 2** and **3**, some degradation of DNA samples is evident; *Mlu*I gave a 3200 kb band with *FCE1A, CRP, APCS* and *SPTA1* on additional gels run under similar conditions, with *APT1A2* hybridized to a 1650 kb *Mlu*I fragment.

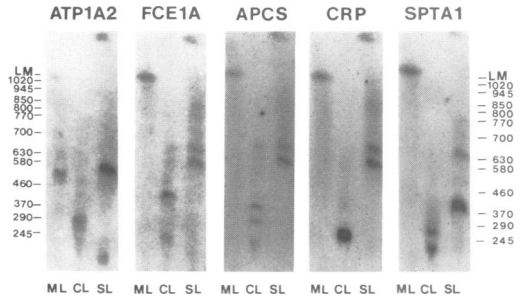

Fig. 6. A human pulsed field gel Southern blot sequentially hybridized with *ATP1A2, FCE1A, CRP, APCS* and *SPTA1* probes. Human PBL DNA was separated by PFGE using ramped pulses from 70 to 145 s. Probes are indicated above each panel. *Mlu*I (Ml), *Cla*I (Cl) and *Sal*I (Sl) restriction endonucleases are shown below each panel. Molecular size standards are indicated in kb alongside the panels. *FCE1A, APCS* and *CRP* probes hybridized to 550, 610, 780, 860 and 920 kb *Sal*I fragments, while *SPTA1* and *ATP1A2* gave disparate sized bands, indicating that *FCE1A, APCS* and *CRP* are located within 550 kb. LM = limiting mobility.

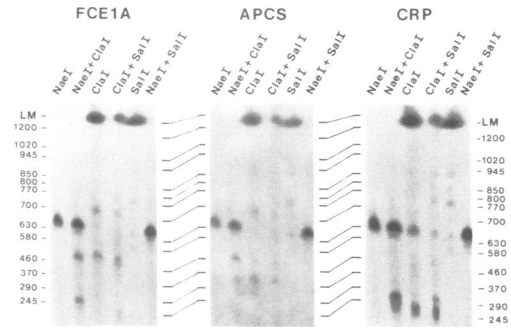

Fig. 7. A human pulsed field gel Southern blot sequentially hybridized with *FCE1A, APCS* and *CRP* probes. Human PBL DNA was separated by PFGE using ramped pulses from 70 to 145 s. Probes are indicated above each panel. Restriction endonucleases are shown above each panel. Molecular size standards are indicated in kb alongside the panels. Double restriction endonuclease digests were performed sequentially with intervening washes as described in Materials and methods. *FCE1A, APCS* and *CRP* identified common *Nae*I and *Sal*I fragments. *Sal*I/*Nae*I double digest gave a major band of the same size as *Nae*I digestion alone for these probes, indicating these *Nae*I sites to be internal to the *Sal*I sites. *FCE1A* and *APCS* hybridized to a common 700 kb *Cla*I, which *Nae*I cleaved into a 250 kb *Nae*I/*Cla*I fragment with *FCE1A*, and a 500 kb *Nae*I/*Cla*I fragment with *APCS*.

by a distance of 1270−2380 kb (Table IIa), no crossovers were evident between these genes in 428 meiotic events examined (Table I). In contrast, three crossovers were evident in 428 meiotic events in the interval between the family of murine interferon-activated genes (*Ifi202, Ifi203* and *Ifi204*) and *Sap*, which were physically separated by 200−250 kb (Table I; Figure 4a). Comparison of these two adjacent genomic segments indicated that the crossover events were not evenly distributed (χ^2 = 15.273, $P <$ 0.01; one degree of freedom). These data indicate that recombination frequency does not vary solely as a function of physical distance on distal mouse Chr. 1.

Discussion

The development of long-range restriction maps of a large syntenic segment of human Chr. 1q and distal mouse Chr. 1 described in this paper permits a novel analysis of genomic organization within a linkage group conserved over ~80 million years of evolution. In addition, the generation of a precise genetic map of this region of mouse Chr. 1 allows comparison of precise physical and recombinational distances in a higher eukaryote.

Conservation of gene order and intergene distance within a linkage group conserved between human and mouse Chr. 1

The order of genes encoding α3-subunit of Na,K-ATPase, α-subunit of IgE Fc receptor, serum amyloid P-component and α-spectrin was the same in human and mouse (Figure 4). Furthermore, localization of *Crp* adjacent to *Fce1a* and *Sap* on mouse Chr. 1 was consistent with the location of its human homolog on human Chr. 1. In both species, the length of the genomic segment occupied by the five physically linked genes was almost identical and physical distances separating each of these genes were very similar (Table IIa).

Two of the genes exhibiting conserved intergenic distance between human and mouse, serum amyloid P-component and C-reactive protein, are members of the pentraxin gene

Table II. Physical interval separating genes and common-sized restriction fragments on mouse and human Chr. 1

(a)

Genes	Murine interval[a]	Human interval
Atpa-3–Spna-1	2780–3220	2710–3400
Atpa-3–Sap	1270–2380	1100–2360
Atpa-3–Fce1a	1600–1920	940–2410
Fce1a–Sap	0–580	0–550
Fce1a–Spna-1	660–1000	500–1400
Sap–Spna-1	200–880	200–1240

(b)

Gene probe(s)	Restriction endonuclease	Fragment size(s)[a]	
		Mouse	Human
Atpa-3, Fce1a, Sap, Crp, Spna-1	*Nru*I	4500	3400
Atpa-3	*Nru*I	1560	1100
Fce1a, Sap, Crp	*Sal*I	1000,1800,2600	2200,2900
Fce1a, Sap, Crp	*Nae*I	880	660
Fce1a, Sap, Crp, Spna-1	*Mlu*I	3500	3200
Atpa-3	*Mlu*I	270,1800	550,1650
Fce1a, Sap, Crp, Spna-1	*Not*I	4500	2400
Atpa-3	*Not*I	2400	1800

[a]Distances between genes (Table IIa) and major restriction fragment sizes identified by gene probes (Table IIb) are in kb as determined by PFGE.

family. The location of murine *Crp* was defined for the first time in the present report: *Crp* mapped to distal mouse Chr. 1, co-segregated with *Sap* and was linked to the latter within 1000 kb. The homologous human genes, *CRP* and *APCS*, were located within 290 kb on human Chr. 1q23. Juxtaposition of pentraxin genes in human and mouse was not unanticipated since they are believed to be products of gene duplication (Mantzouranis *et al.*, 1985; Ohnishi *et al.*, 1986). More remarkable, however, was interspecies conservation of gene order and intergenic distance evident among the other three genes, which have neither sequence similarity nor regulatory elements in common with one another or with the pentraxins.

Previous studies have shown that the order of many MHC genes are the same in mouse and human (Hardy *et al.*, 1986; Steinmetz *et al.*, 1986; Nadler *et al.*, 1987). Three genes have recently been physically linked in the same order within 600 kb on murine (Brockdorff *et al.*, 1989) and human Chr. X (Patterson *et al.*, 1987). The current study demonstrates that genomic conservation is not limited to gene families or special regions of the genome such as the MHC or sex chromosomes. In addition, we have identified two further autosomal 6000 kb segments, each comprising six genes, for which gene order and intergenic distance are the same in human and mouse (S.F.Kingsmore and M.F.Seldin, unpublished data). Collectively these date strongly imply that conservation of gene order and intergenic distance are characteristic of many linkage groups conserved between human and mouse.

Conservation of unmethylated CpG dinucleotides

Conservation of unmethylated CpG residues between human and mouse is suggested by marked similarities in the distribution of cleavage sites for the restriction endonucleases *Not*I, *Mlu*I, *Sal*I, *Nru*I and *Nae*I evident in this region (Table IIb). These restriction endonucleases recognize sequences rich in unmethylated CpG dinucleotides, which occur infrequently in vertebrate genomes and tend to be clustered as CpG-islands (Bird, 1986). Since *Mlu*I and *Not*I complete digests yielded unusually large fragments in both human and mouse with gene probes specific for the IgE Fc receptor α-subunit, serum amyloid P-component, C-reactive protein and α-spectrin (Table IIb), it is unlikely that observed interspecific similarities in restriction maps of this region are coincidental.

Cleavage sites for several restriction enzymes (*Not*I, *Nae*I, *Sac*II, *Nru*I and *Bss*HII) were, within experimental error, coincident (Figure 4), which is characteristic of CpG-islands at these sites (Brown and Bird, 1986; Lindsay and Bird, 1987). Some of these putative 'CpG-islands' appear to have been conserved between human and mouse genomes, such as the 'CpG-island' containing *Not*I and *Mlu*I sites which separated the α3-subunit of Na,K-ATPase and IgE Fc receptor α-subunit genes, and the *Sal*I/*Nae*I 'CpG-island' between the pentraxin genes and α-spectrin (Figure 4a and b). This is the first report of comparative mapping of 'CpG-islands' in disparate species. Further studies will be necessary to determine the extent and significance of conservation of specific CpG-rich restriction endonuclease sites and/or CpG-islands between human and mouse.

Comparison of physical and genetic maps of a segment of distal mouse Chr. 1

Some aspects of the relationship between genetic and physical chromosome maps have been examined in lower eukaryotes such as *Drosophila melanogaster* and *Saccharomyces cerevisiae*. In these organisms, the frequency of meiotic recombination is altered by the proximity of chromosomal structural elements, such as the centromere (Dobzhansky, 1930; Symington and Petes, 1988), and by specific sequences which stimulate homologous recombination locally, such as *cog* in *Neurospora* and M26 in *Schizosaccharomyces pombe* (Angel *et al.*, 1970; Gutz, 1971), which are similar to Chi sites in *Escherichia coli* (Stahl and Stahl, 1977). In mammalian genomes, however, little is known concerning the relationship between genetic and physical distances. Generation of precise physical and genetic maps (the latter based upon recombination frequencies in 428 [(C3H/HeJ-

gld/gld × *M.spretus*)F1 × C3H/HeJ-*gld/gld*] backcross mice) of *Atpa-3, Fce1a, Sap, Crp, Ifi202, Ifi203, Ifi204* and *Spna-1* gene probes permits comparison of genetic and physical distances within a 2780−3220 kb (0.7 cM) interval of mouse Chr. 1. In comparison with an average ratio of 1 cM = 2000 kb (Barlow and Lehrach, 1987), the region *Atpa-3*−*Spna-1* is a relative coldspot for meiotic recombination, containing only three crossovers in 428 meiotic events. Furthermore, crossovers were not uniformly distributed within this region. No crossovers occurred in the 1270−2380 kb interval between *Atpa-3* and *Sap*, while all three crossovers (significantly more than expected) were observed within the 200−250 kb interval between *Sap* and the interferon-activated gene family. Thus the interval between *Atpa-3* and *Sap* appears to represent a coldspot and the interval between *Sap* and the interferon-activated genes a hotspot for crossover events. These data are consistent with previous observations that certain segments of mammalian genomes, such as the human pseudoautosomal region and parts of the murine and human MHC, exhibit dramatically elevated recombination rates (Steinmetz *et al.*, 1986, 1987; Goodfellow *et al.*, 1986). Thus non-uniformity of distribution of crossovers along mammalian chromosomes appears to be a general phenomenon.

The present data also raise the possibility that crossover frequency within mammalian chromosomal segments may be related to the presence of CpG-islands. For example, the interval between *Atpa-3* and *Sap*, which is a recombinational coldspot, was CpG poor as evidenced by extremely large *Not*I and *Mlu*I fragments (Figure 4a). However, the recombinational hotspot between *Sap* and *Ifi202/Ifi204* contained a prominent CpG-island (Figure 4a). Previous physical mapping studies of the pseudoautosomal region of human Chr. X, which is a recombinational hotspot by virtue of a single obligatory crossover during male meiosis, have demonstrated a high density of CpG-islands within this 3000 kb region (Brown, 1988; Petit *et al.*, 1988; Rappold and Lehrach, (1988). Sequencing of a probable hotspot for meiotic recombination within the mouse MHC showed an excess of GC dinucleotides (Steinmetz *et al.*, 1986). Physical mapping of a 12 000 kb region of human Chr. 7 around the cystic fibrosis locus revealed a paucity of CpG-islands, which correlated with a recombinational coldspot in this region (Fulton *et al.*, 1989). The physical association of CpG-islands with crossover sites suggests the possibility that CpG-islands may function as a vertebrate equivalent of Chi-sequences. Molecular cloning of the interval between *Sap* and *Ifi202/Ifi204* will determine the exact location of these crossover events and permit analysis of their relationship to CpG-islands.

Significance of conservation of genomic organization within syntenic groups

The present report suggests that within a large segment of a conserved linkage group gene order, intergenic distance and unmethylated CpG dinucleotides have remained relatively unchanged over 80 million years of evolution. Previous studies have amply demonstrated exonic conservation over similar periods (King and Wilson, 1975; Curtis *et al.*, 1985; Whitehead *et al.*, 1988). Collectively, these data imply that mammalian genomes are relatively inert structures over evolutionary time, punctuated by infrequent chromosomal rearrangements. Conservation of chromosomal organization has profound theoretical and practical consequences: chromosomal rearrangement events provide a cogent mechanism for punctuated molecular evolution. Chromosomal translocations, inversions, duplications and deletions act as sterility barriers which may facilitate speciation (Wilson *et al.*, 1977), and, by divorcing genes or gene clusters from flanking regulatory sequences and juxtaposing them adjacent to novel regulatory elements, could create species diversity (King and Wilson, 1975).

Conservation of genomic organization within syntenic groups also has practical consequences for strategies to map the human genome and for development of true mouse genetic models of human hereditary diseases. Genetic defects which lead to the development of disorders in experimental animals, once mapped and cloned, may predict the location of a human homolog and lead to an understanding of the genetic basis of the human disorder (Lalley *et al.*, 1988).

Precise genetic mapping in the mouse is facilitated by the ability to examine very large numbers of meiotic events and by ease of detection of informative RFLPs using interspecific backcrosses. Precise genetic mapping in the mouse of genes suspected of being localized within a conserved segment can provide a contingent order for homologous human genes, which may then be linked using PFGE. In the present report, phsyical linkage of *ATP1A2, FCE1A, APCS, CRP* and *SPTA1*, which had previously been localized to a large region of human Chr. 1q (Table I), was accomplished by such a comparative mapping approach. Furthermore, we have utilized this strategy to develop physical maps of two additional 6000 kb segments of human Chr. 1q and 1p (S.F. Kingsmore and M.F.Seldin, unpublished data). These studies should assist efforts to establish a physical map of the human genome, identify molecular principles governing meiotic recombination and, perhaps, define chromosomal rearrangement events underlying mammalian evolution.

Materials and methods

Mice

C3H/HeJ-*gld/gld* and *M.spretus* (Spanish) mice and [C3H/HeJ-*gld/gld* × *M.spretus*)F1 × C3H/HeJ-*gld/gld*] backcross mice were bred and maintained as previously described (Seldin *et al.*, 1988a).

Southern hybridization

DNA isolated from mouse organs by standard techniques was digested with restriction endonucleases and 10 μg samples were subjected to electrophoresis in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher and Schuell Inc., Keene, NH), hybridized at 65°C and washed under stringent conditions, all as previously described (Seldin *et al.*, 1988a).

Pulsed field electrophoresis

Preparation of high mol. wt DNA in agarose blocks, restriction enzyme digestion, PFGE and Southern transfer were performed as previously described (Kingsmore *et al.*, 1989b). In brief, viable murine lymphocytes, purified from teased C3H/HeJ-*gld/gld* lymph nodes, and human peripheral mononuclear cells were resuspended in 0.5% low-melting point agarose (FMC BioProducts, Rockland, ME) at 6×10^5 cells per 40 μl block. (NB: no differences in restriction fragment sizes were observed using DNA from various mouse organs or different inbred laboratory strains with the gene probes used in this study.) DNA was prepared by incubation of agarose blocks in 0.5 M EDTA (pH 9.0), 1% sodium lauroyl sarcosinate, 2% proteinase K at 50°C twice for 48 h. Blocks were then washed three times with 10 mM Tris, 1 mM EDTA, pH 8.0 (TE), twice with TE plus 0.04 mg/ml phenylmethylsulfonylfluoride, three times with TE and stored at 4°C. After equilibration with appropriate buffer, DNA samples were digested with 2−10 units/μg DNA of restriction endonucleases (Boehringer Mannheim Biochemicals, Indianapolis, IN) for 4−16 h. Double enzyme digests were performed sequentially with intervening washes as above. Following digestion, blocks were equilibrated with 0.25 M EDTA and loaded

into 1% agarose gels. PFGE was carried out at 14°C in X1 TBE using a Pulsaphor unit (Pharmacia-LKB, Piscataway, NJ). Separation of 50–1300 kb DNA molecules was achieved using pulses ramped continuously from 70 to 145 s at 180 V for 46 h; 800–6000 kb DNA was resolved by ramped pulses of 15–90 min at 50 V for 8 days. Gels were stained with ethidium bromide to visualize molecular size standards (oligomers of lambda phage, and chromosomes of *S.cerevisiae* and *S.pombe*; FMC BioProducts), which were marked with india ink. After UV exposure for 2 min, gels were incubated in 0.5 M NaOH, 1.5 M NaCl for 1 h and blotted in the same solution onto GeneScreen membranes (NEN, Boston, MA) for 48 h. Filters were then neutralized in 50 mM Na phosphate (pH 6.5), baked, and cross-linked using 300 μW/cm^2 254 nm UV light for 2 min. Filter hybridizations were carried out as previously described (Kingsmore *et al.*, 1989a). Assignment of two probes to a common restriction fragment was based on several criteria: sequential hybridization of a filter, and exhibition of identity by double or partial digests.

Molecular probes

All probes were labeled by the hexanucleotide technique with [α-^{32}P]dCTP as previously described (Seldin *et al.*, 1988a). Gene probes used were: for *Ly-17* (*CD32*), a 1.3 kb insert from mouse cDNA clone Fc$_\gamma$Rα (Seldin *et al.*, 1988b); for *Atpa-3*, a 3 kb *Eco*RI fragment from rat cDNA clone z13C (Kent *et al.*, 1987); for *Fce1a*, a 600 bp *Sty*I rat cDNA clone (Kinet *et al.*, 1987) or a 1.0 kb mouse cDNA clone (kind gift of Dr J.-P.Kinet); for *Sap*, mouse cDNA clone MSAP5 (Whitehead *et al.*, 1988); for *Ifi202* (provisional gene designation), a 170 bp *Pst*I/*Eco*RI insert from genomic clone 202-exon 1 (Kingsmore *et al.*, 1989b); for *Ifi203* (provisional gene designation), a 1.1 kb insert from mouse cDNA clone 203.2 (Kingsmore *et al.*, 1989b); for *Ifi204* (provisional gene designation), mouse cDNA clone 17b (Kingsmore *et al.*, 1989b); for *Spna-1*, a 750 bp *Pst*I insert from mouse cDNA clone pMaSp1 (Curtis *et al.*, 1985); for *D1Pas1* (provisional gene designation), mouse cDNA clone Pl-10 (Kingsmore *et al.*, 1989a); for *ATP1A2*, a 2.7 kb *Eco*RI insert from human cDNA clone Huaα3p (kind gift of Dr Dackowski, Integrated Genetics, Inc); for *FCE1A*, a 1.1 kb *Xba*I/*Sac*I fragment of the human cDNA clone 110b-1 (Tepler *et al.*, 1989); for *APCS*, human cDNA clone pSAP11 (Mantzouranis *et al.*, 1985); for *CRP* and *Crp* (provisional gene designation), a 1.9 kb *Pst*I insert from the human cDNA clone pCRP5 (Woo *et al.*, 1985); for *SPTA1*, a 700 bp *Pst*I insert from human cDNA clone pHαSp6 (Heubner *et al.*, 1985).

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