Brief Definitive Report

GENETIC ORGANIZATION OF COMPLEMENT RECEPTOR-RELATED GENES IN THE MOUSE

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The human genes encoding the complement regulatory proteins factor H (*CFH*), C4-binding protein (*C4BP*), complement receptors type 1 (*CR1*) and type 2 (*CR2*), decay-accelerating factor (*DAF*), and membrane cofactor protein (*MCP*) form an important linkage group, designated the regulator of complement activation (*RCA*) locus. In addition to complementary functions, members of the *RCA* gene family share a repetitive protein domain of 60 amino acids. *RCA* locus genes have been mapped to human chromosome (Chr.) 1q32 by in situ hybridization (1-4). Furthermore, the human genes encoding *CR1*, *CR2*, *DAF*, and *C4BP* are clustered in that order within 750 kb of genomic DNA (5, 6).

Several murine homologues of the *RCA* gene family have recently been cloned, including cDNAs for murine factor H (*Cfh*), C4-binding protein (*C4bp*), and the proposed murine homologues of *CR1* (*Mcry*) and *CR2* (*Mcr2*) (7-11). These murine *RCA* genes exhibit considerable sequence homology with their human counterparts, including the repeated consensus sequences, and all map to mouse Chr. 1 (9-11). However, the genetic organization of the murine *RCA* gene family has not been delineated.

Previous studies have shown a large linkage group is conserved between human Chr.1q21-q32 and distal mouse Chr.1 (12-14). By comparative mapping studies using DNA from a large panel of intraspecific backcross mice, 10 genes, including murine *C4bp*, have been located within this syntenic group; all appear to be arranged colinearly in man and mouse (14). The current study defines the genetic organization of members of the murine *RCA* gene family on distal mouse Chr.1. We report that murine *C4bp* and *Cfh* map within the conserved linage group, but *Mcry* and *Mcr2*, while closely linked to each other, are located 40 cM telomeric to *C4bp*, outside the conserved group. These studies suggest that a translocation or inversion within the *RCA* family occurred during the evolution of the mouse and may in part explain evolutionary divergence of complement receptor-related genes.

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Materials and Methods

Mice. C3H/HeJ-*gld/gld* and *Mus spretus* (Spanish) mice and ([C3H/HeJ-*gld/gld* × *M. spretus*] F_1 × C3H/HeJ-*gld/gld*) backcross mice were bred and maintained as previously described (12).

Southern Hybridization. DNA isolated from mouse organs by standard techniques was digested with restriction endonucleases and 10-μg samples were subjected to eletrophoresis in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher & Schuell, Inc., Keene, NH), hybridized at 65°C, and washed under stringent conditions, all as previously described (12).

Molecular Probes. All probes were labeled by the hexanucleotide technique with α-[^{32}P]dCTP as previously described (12). *C4bp* RFLP were identified using mouse cDNA clone pMBP.15 (8); *Cfh* RFLP with a 1.45-kb Bam HI fragment subcloned from *Cfh* cDNA including exons 8–15 (7); *Mcry* RFLP were detected with the cDNA clone MScR1.0 (9, 10); and *Mcr2* RFLP were detected with clones 107-1 and cDNA-1 obtained from a mouse spleen cDNA library screened with human *CR2* clone. The 107-1 and cDNA-1 probes are very homologous to human *CR2* cDNA sequences and identify a mRNA species very similar in size and tissue distribution as those seen with human *CR2* (11). *Pl-10* was obtained from a testes-specific cDNA library and was found by in situ hybridization to localize to the telomeric region of mouse Chr.1 (44% of silver grains with 88% to the H band) and to Chr.X (36% of silver grains with 75% to A2-A3 bands) (M. G. Mattei and P. Leroy, unpublished results).

Results and Discussion

Murine genes were mapped by linkage analysis of RFLP in genomic DNA samples generated from ([C3H-*gld/gld* × *M. spretus*]F_1 × C3H-*gld/gld*) backcross mice. RFLP were determined by Southern blot hybridization of DNA from C3H-*gld/gld* parental mice and (C3H-*gld/gld* × *M. spretus*)F_1 mice digested with various restriction endonucleases. *M. spretus* was chosen as the second parent because of the relative ease of detection of informative RFLP in comparison with crosses using conventional

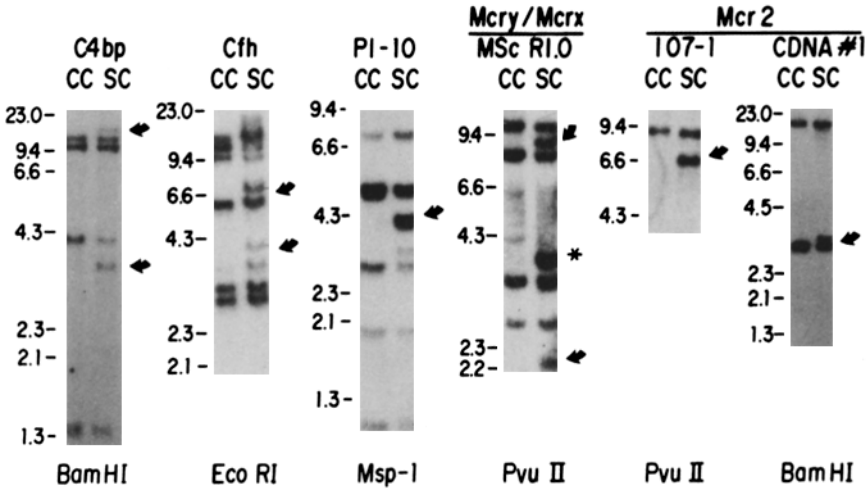

FIGURE 1. Southern blot identification of unique *M. spretus* RFLP detected with mouse *RCA* molecular probes and an unrelated gene *Pl-10*. The gene probes indicated at the top of the figure are described in Materials and Methods. Restriction endonucleases are indicated at the bottom and molecular size standards (in kilobases) are shown at the left of each panel. Arrows signify bands present in DNA from (C3H-*gld/gld* × *M. spretus*)F_1 (SC) but not in C3H-*gld/gld* (CC) mice. For MScR1.0, the arrows indicate *Mcry* RFLP and the star indicates a *Mcrx* RFLP.

TABLE I

Recombination Events among Markers of Distal Mouse Chromosome 1 in 200 (C3H/HeJ-gld/gld × M. spretus) × C3H/HeJ-gld/gld Backcross Mice

Marker	*C4bp*	*Ren-1,2*	*CD45*	*Cfh*	*Lamb-2*	*Abll*	*At-3*	*Apoa-2/ CD32*	*Spna-1*	*Pl-10*
Ren-1,2	3	-	17	18	30	32	38	55	59	70
CD45	17	14	-	1	13	15	21	38	42	53
Cfh	18	15	1	-	12	14	20	37	41	52
Lamb-2	30	27	13	12	-	2	8	25	29	40
Abll	32	29	15	14	2	-	6	23	27	38
At-3	38	35	21	20	8	6	-	17	21	32
*Apoa-2/CD32**	55	52	38	37	25	23	17	-	4	15
Spna-1	59	56	42	41	29	27	21	4	-	11
Pl-10	70	67	53	52	40	38	32	15	11	-
*Mcry/Mcr2**	80	77	63	62	50	48	42	25	21	10

Genotypes for markers were determined by RFLPs illustrated in Fig. 1 and references 11 and 13. Numbers indicate individuals recombinant for a pair of markers of 200 backcross mice typed. With the gene order given, no double or multiple crossovers were seen.

* No recombinants evident in 200 backcross mice.

inbred strains. Fig. 1 shows unique RFLP (*M. spretus*) present in the F_1 mice for *C4bp*, *Cfh*, *Mcry*, *Mcr2*, and *Pl-10* gene probes. 200 backcross mice were typed by segregation analysis of these RFLP and also by previously described RFLP detected with probes for the genes *Ren-1,2*, *CD45*, *Lamb-2*, *Abl1*, *At-3*, *CD32* (formerly *Ly-17*), *Apoa-2*, and *Spna-1* (12–14). At each locus, mice displayed either the homozygous C3H (CC) or the heterozygous F_1 pattern (SC). Gene order was established by minimization of chromosome crossover events. The gene order given in Table I resulted in elimination of double crossover events and was unambiguous.

The RFLP associated with *Pl-10* maps 5.5 cM telomeric to *Spna-1* (Table I), the most telomeric member of the conserved linkage group, and was placed 0.8 cM telomeric of *Akp-1* (14, data not shown). In situ hybridization of human chromosomes with *Pl-10* failed to detect a homologous sequence on Chr.1, but rather hybridized predominantly to 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). Mapping of *Pl-10* outside the conserved linkage group is consistent with previous results defining the end point of the conserved group within human Chr.1q21 (12, 13).

Segregation analysis of the backcross mice revealed that murine *C4bp* maps 1.5 cM centromeric to *Ren-1,2* (Table I). This location is consistent with the relative positions of their homologues on human Chr.1 (Fig. 2). *Cfh* is located 0.5 cM telomeric to *CD45*, and 9 cM telomeric to *C4bp* (Table I). This finding is also in agreement with linkage data in man, where *C4BP* and *CFH* are 6.9 cM apart (15), and could not be linked by pulsed field electrophoresis (5, 6). Interestingly, *Cfh* shares less sequence homology than other members of the *RCA* gene cluster. Together, these data suggest that *Cfh* may have diverged relatively early in evolution from other *RCA* genes.

Although tightly linked, with no recombinations evident in 200 meiotic events, *Mcry* and *Mcr2* map outside the conserved linkage group, 40 cM telomeric to *C4bp* on mouse Chr.1 (Table I). The tight linkage of *Mcry* and *Mcr2* is in accord with physical

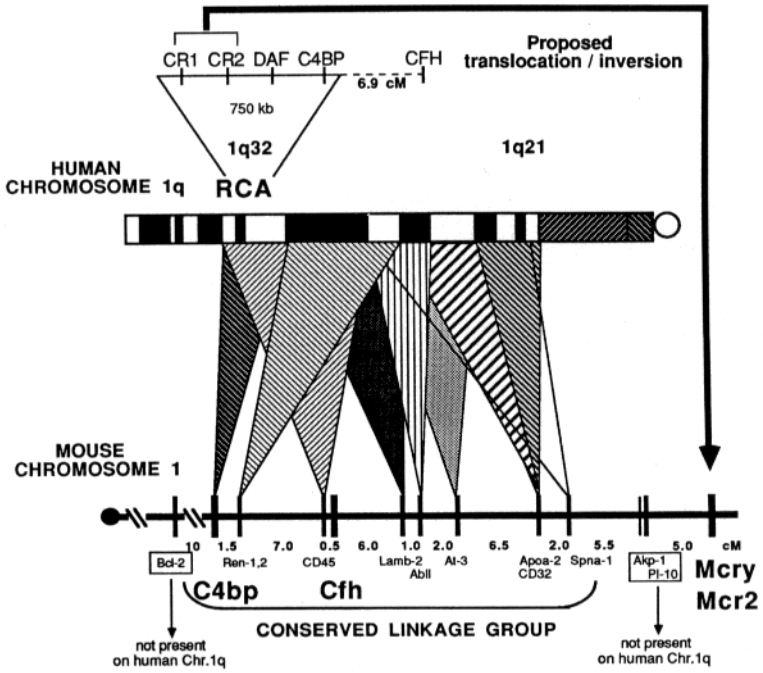

FIGURE 2. Gene linkage relationships among members of the murine and human *RCA* family. Murine map distances (in centimorgans) are based on 200 meiotic events in backcross mice (see Table I). Syntenic relationships are indicated by wedges connecting mouse genes with their human homologues. Localization of human *RCA* genes is based on in situ hybridization and genetic studies (1–6, 14). Positions of other human genes, based on in situ hybridization, are: *REN* 1q25-31, *CD45* 1q31-32, *LAMB2* 1q25, *ABLL* 1q24-25, *AT3* 1q23-25, *APOA2* 1q21-23, *CD32* 1q23-24, *SPTA* 1q21.3-25 (cited in reference 14). Mouse genes centromeric to *C4bp* are not members of this conserved linkage group nor are human genes centromeric of human Chr.1q21.

mapping studies, which indicate that *Mcry* and *Mcr2* are contiguous genes, located within 10 kb of one another (11), and with the physical mapping of *CR1* and *CR2* to within 75 kb in man (5, 6). However, the mapping of *Mcry* and *Mcr2* 40 cM telomeric to *C4bp* on mouse Chr.1 is not syntenic with the position of *CR1* and *CR2* on human Chr.1 (Fig. 2). Assuming that *Mcry* and *Mcr2* are indeed the murine homologues of *CR1* and *CR2*, they define a breakpoint in the large conserved linkage group between distal mouse Chr.1 and human Chr.1q21-32, and suggest that a translocation or inversion occurred within the *RCA* gene family during the evolution of the mouse (Fig. 2). The mapping of *Pl-10*, which is not a member of the conserved linkage group, centromeric to *Mcry* and *Mcr2*, supports this hypothesis (Fig. 2). In addition, *C4bp* is the most centromeric member of the conserved linkage group thus far identified (Fig. 2).

Several lines of evidence suggest that *Mcry* and *Mcr2* are the murine homologues of *CR1* and *CR2*. Only *Mcry* and *Mcr2* sequences were detected upon hybridization of murine cDNA libraries with human *CR1* and *CR2* cDNA probes, respectively, with the exception of *Mcrx*, an *Mcry*-related pseudogene, which maps to mouse Chr.8 (9–11). Screening of human cDNA and genomic DNA libraries with *Mcry* cDNA

probes only identified *CR1* sequences (10). Sequence analysis of *Mcry* confirmed the presence of the characteristic consensus repeat sequences (10). However, differences have been found between *Mcry* and *CR1*, and between *Mcr2* and *CR2*, with respect to distribution of gene expression and molecular size of their mRNA transcripts (9–11). Murine homologues of *DAF*, *MCP*, and *F13B* genes have not been identified. No crosshybridization was evident on mouse Southern blots probed at low stringency with human *DAF*, *MCP*, or *F13B* (data not shown). While *Mcry* and *Mcr2* represent those murine sequences most homologous to *CR1* and *CR2*, it is suggested that divergent evolution, subsequent to a translocation or inversion event within the *RCA* ancestor, may have resulted in *Mcry* and *Mcr2* gene products fulfilling roles of *CR1*, *CR2*, *DAF*, and *MCP*.

The gene linkage results reported here extend the genetic map of mouse Chr.1 by the addition of three new telomeric markers and, more importantly, provide additional insight into the evolution of complex genomes. These studies raise an intriguing hypothesis for the evolutionary basis for diversification of gene structure and function: evolution of higher eukaryotes was punctuated by large changes in genomic organization, which allowed subsequent dramatic divergence of "homologous" genetic units. Comparative studies in additional species may allow further analysis of both the evolution of genomic organization and its relationship to functional divergence. The *RCA* family may serve as a model for such studies.

Summary

Using an interspecific cross, gene linkage relationships among members of the murine complement receptor-related genes, *C4bp*, *Cfh*, *Mcry*, and *Mcr2*, were analyzed by segregation of RFLP in 200 mice. The human homologues of these genes are tightly linked, composing the *RCA* locus, which maps to human chromosome (Chr.)1q32, within a large linkage group conserved between human Chr.1q21-32 and mouse Chr.1. RFLP associated with *C4bp* and *Cfh* map within this conserved linkage group; *Cfh* is located 9 cM telomeric to *C4bp*, which is consistent with linkage data for their human homologues. *Mcry* and *Mcr2*, while tightly linked, are located outside the conserved group, 40 cM telomeric to *C4bp*. These data suggest that a translocation or inversion occurred within the *RCA* family during the evolution of the mouse, defining a breakpoint of this large conserved linkage group.

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