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 linkage 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] F1 × 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 electrophoresis 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-[32P]dCTP as previously described (12). C4bp RFLP were identified using mouse cDNA clone pMBP.15 (8); C4a RFLP with a 1.45-kb Bam HI fragment subcloned from C4a cDNA including exons 8–15 (7); Mcry RFLP were detected with the cDNA clone MScR1.0 (9, 10); and Mcrr2 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). P140 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]F1 × 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)F1 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

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inbred strains. Fig. 1 shows unique RFLP (M. spreitus) present in the F1 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, Abll, 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 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 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
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 Mrex, 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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