Brief Definitive Report

THE GENE ENCODING DECAY-ACCELERATING FACTOR (DAF) IS LOCATED IN THE COMPLEMENT-REGULATORY LOCUS ON THE LONG ARM OF CHROMOSOME 1

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To focus complement attack on foreign substances and avoid damage to host tissues, steps in the complement cascade are controlled by specific regulatory factors. In one such step, C3 convertase assembly, both membrane and serum regulatory proteins participate (reviewed in 1 and 2). Each of the membrane regulatory proteins [C3b/C4b receptor (CR1), decay-accelerating factor (DAF), and membrane cofactor protein (MCP)] and each of the serum regulatory proteins [factor H (H), and C4-binding protein (C4bp)] binds to one or both of the homologous proteins, C3b and C4b. This similarity of function prompted the suggestion that some or all of these proteins may have arisen from an ancestral C3b/C4b-binding protein (2). Segregation studies of polymorphisms of CR1, H, and C4bp in families established that the genes for these three complement-regulatory proteins are tightly linked in man (3). Nucleotide sequence analyses of cDNAs encoding the three proteins (4–6) revealed that each is made up of variable numbers of contiguous ~60-amino-acid-long repeating units containing conserved cys, pro, gly, trp, leu/ile/val, and tyr/phe residues. In situ hybridization studies (4) localized the human *CR1* gene (and hence the CR1-C4bp-H linkage group) to the long arm of chromosome 1 at band 1q32.

DAF, first described by Hoffmann (7), was isolated and characterized by Nicholson-Weller et al. (8). We have recently cloned and sequenced the cDNA for human DAF (9). The derived protein sequence displayed four of the 60-amino-acid repeating units. Since DAF thus contains structural as well as functional homologies to the CR1-C4bp-H complement-regulatory linkage group, we undertook an investigation of the chromosomal location of DAF by analysis of

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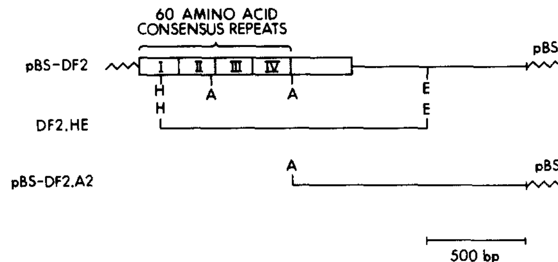

FIGURE 1. DAF probes used in chromosomal localization study. pBS-DF2 is the nearly full-length DAF cDNA clone in the plasmid pBS (9); the open boxes show the coding region, and the four contiguous domains that code for the 60-amino-acid consensus repeats are marked by Roman numerals. Sites for the restriction endonucleases Hind III (*H*), Ava II (*A*), and Eco RV (*E*) are shown. DF2.HE is the Hind III–Eco RV fragment of pBS-DF2, and pBS-DF2.A2 is the fragment downstream from the second Ava II site.

segregation patterns in somatic cell hybrids and by in situ hybridization to human metaphase chromosomes.

Materials and Methods

Somatic Cell Hybrids. Somatic cell hybrids were formed by PEG 1,000–mediated fusion of human VA2, A549, or IMR90 fibroblast cells to Chinese E36 or Syrian BHK-B1 hamster cells as previously described (10). The human chromosomal composition of the hybrid clones was assessed by screening for up to 34 gene-enzyme systems (11), and in selected cases by complete cytogenetic analyses using trypsin-Giemsa banding (12). High-molecular-weight DNA from these clones was digested to completion with Eco RI restriction endonuclease (New England Biolabs, Beverly, MA), separated by electrophoresis through an agarose gel, transferred by Southern blotting to nitrocellulose or nylon membranes, and hybridized with a ^{32}P DAF cDNA probe (9) labeled by the random oligonucleotide priming method to a specific activity of 1–3 $\times 10^9$ dpm/μg.

In Situ Chromosomal Hybridization. Human metaphase cells prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes were hybridized with ^{3}H-labeled DAF cDNA probes. Radiolabeled probes were prepared by nick translation of the entire plasmid with all four ^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.0 $\times 10^8$ dpm/μg. In situ hybridizations were performed as previously described (13). Metaphase cells were hybridized at 4.0 and 8.0 ng of probe per milliliter of hybridization mixture. Autoradiographs were exposed for 11 d.

Results and Discussion

Southern blots of Eco RI digests of DNA from 53 hamster-human somatic cell hybrids were hybridized with ^{32}P-labeled DAF cDNA probe DF2.HE (Fig. 1). This identified several Eco RI fragments from human DNA (Fig. 2). The full panel of somatic cell hybrids was analyzed for discordance of the *DAF* gene and specific human chromosomes, i.e., percent asynteny (Table I). The *DAF* gene is located on chromosome 1; all other possible chromosome localizations were associated with a minimum of six discordant clones. Interestingly, one clone lacked a human *DAF* gene by blotting, lacked an identifiable human chromosome 1 by cytogenetic analysis, failed to express the gene-enzyme PEPC, located at 1q24 or 1q42, but did express PGD, localized to 1p36.2-p36.13, and PGM1, located at 1p22.1. The cytogenetic analysis did not exclude the possibility of a portion of 1p being present either as a fragment or translocated to a human or hamster chromosome. This result suggests that the *DAF* gene was located either on the long arm of chromosome 1 or near the centromeric region.

To confirm the association of DAF with chromosome 1 (using an independent technique) and to sublocalize the *DAF* gene on chromosome 1, in situ hybridi-

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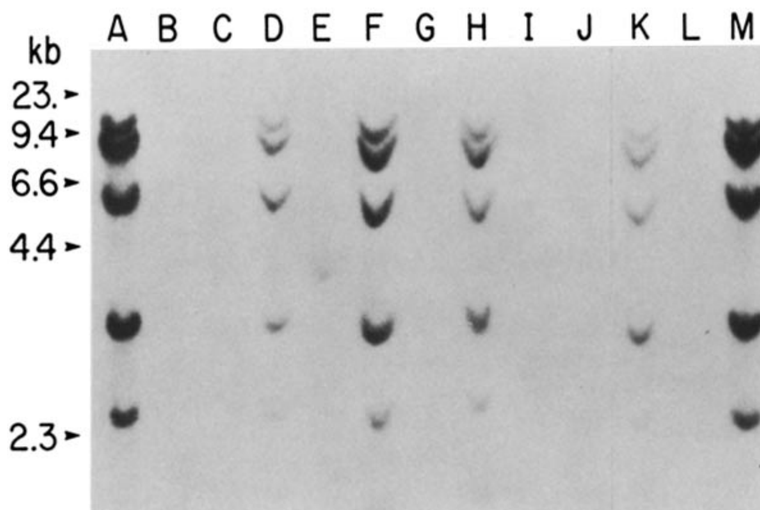

FIGURE 2. Hybridization of DAF-specific probe to DNA from hamster × human somatic cell hybrid clones. Human and hamster control DNAs are in lanes *A* and *B*, respectively. DNA from hybrid clones containing human chromosome 1 are in lanes *D, F, H, K,* and *M*; DNA from hybrids lacking chromosome 1 are in lanes *C, E, G, I, J,* and *L*. DF2.HE probe (Fig. 1) was hybridized to Southern blots of Eco RI digests as described in Materials and Methods.

zations to normal human metaphase chromosomes were performed using two different DAF probes. These hybridizations (repeated twice with similar results) produced specific labeling only of human chromosome 1. In hybridizations performed with the full-length cDNA probe (pBS-DF2), we observed specific labeling of the distal part of the long arm of chromosome 1. Of 100 metaphase cells examined from this hybridization, 40 (40%) were labeled on region q3 or q4, bands q31-41 of one or both chromosome 1 homologs ($p < 0.0005$). The distribution of labeled sites on chromosome 1 is illustrated in Fig. 3*A*. A total of 62 grains was observed on this chromosome; of these 45 (73%) were clustered at bands q31-41 and represented 28% (45 of 161) of all labeled sites. The largest cluster of grains was observed at 1q32.

The pBS-DF2 cDNA clone contains four copies of the homologous repeating unit characteristic of the C3b-binding gene family. To eliminate the possibility that the specific labeling of 1q31-41 observed in this hybridization was due to crosshybridization to homologous repeating units of related genes, which also map to this region of chromosome 1 (3, 4, 14), we hybridized the pBS-DF2.A2 probe to normal metaphase cells. The latter probe is an 1,150-bp Ava II fragment of the pBS-DF2 cDNA clone, and does not contain the repeating units (Fig. 1). We again noted specific labeling only of chromosome 1. We examined 100 metaphase cells and the distribution of labeled sites on this chromosome is illustrated in Fig. 3*B*. Of these, 38 cells were labeled on region q3 or q4 (bands q31-41) of one or both chromosome 1 homologs. A total of 60 grains was observed on this chromosome; of these 43 (72%) were clustered at bands 1q31-41 and represented 23% (43 of 185) of all labeled sites ($p < 0.0005$). The largest cluster of grains was again observed at 1q32. Thus, the *DAF* gene is localized to human chromosome 1, at bands q31-41.

Mapping DAF to the same locus as the genes for CR1, CR2, factor H, and

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TABLE I

Synteny Test of DAF Gene and Human Chromosomes in Rodent × Human Hybrid Clones

Human chromosome	DAF gene/human chromosome				Asynteny (%)
	+/+	+/−	−/+	−/−	
1	22	0	1*	30	2
2	9	11	3	19	33
3	0	6	1	3	70
4	5	5	5	14	34
5	8	7	11	9	51
6	14	7	16	15	44
7	2	7	1	5	53
8	10	9	9	13	44
9	5	4	4	7	40
10	5	5	8	11	44
11	17	1	21	6	49
12	12	4	17	11	48
13	11	3	11	1	54
14	15	5	18	14	44
15	10	8	12	7	54
16	8	3	9	5	48
17	17	0	11	0	39
18	2	7	0	8	41
19	10	9	6	7	47
20	8	5	12	7	53
21	1	6	5	6	61
22	0	6	0	5	55
X	7	4	15	6	59

Somatic cell hybrids were scored for the presence (+) or absence (−) of specific human chromosomes as described in Materials and Methods and for the presence or absence of DAF coding sequences by Southern blot hybridization.

* This hybrid clone expresses two gene-enzymes located on chromosome 1p, does not express a gene-enzyme marker on 1q, and does not have a human chromosome 1 identifiable by cytogenetic analysis.

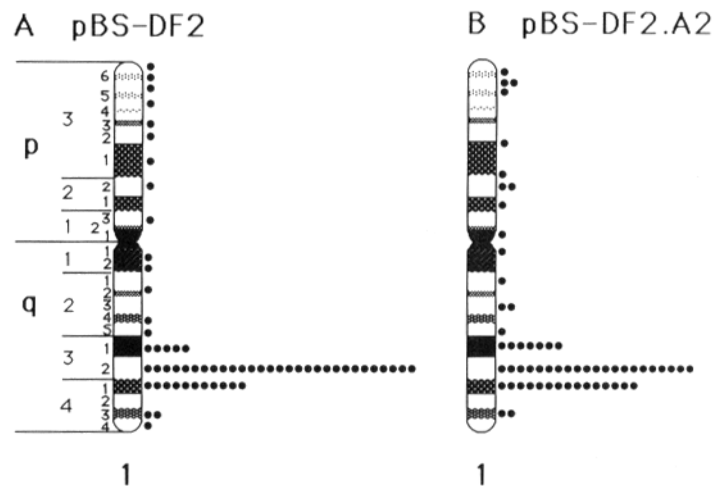

FIGURE 3. Distribution of labeled sites on chromosome 1 in 100 normal metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes that were hybridized with the pBS-DF2 (*A*) or pBS-DF2.A2 (*B*) probes. The labeled sites observed in these hybridizations were clustered in 1q31-41; the largest cluster of grains was located at 1q32.

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C4bp (3, 4, 14) adds a fifth member to this gene cluster which codes for C3 regulatory and receptor proteins. The relation of DAF and the other proteins in this chromosome 1 linkage group to the larger superfamily of proteins that contain 60-amino-acid consensus repeats is not yet clear (15). The proteins in the superfamily divide functionally into three categories: (*a*) complement-regulatory proteins, including DAF; (*b*) complement activation proteins that bind C3 or C4 fragments, including C1r, C1s, C2, and factor B; (*c*) noncomplement proteins, including the IL-2 receptor, coagulation factor XIII, and β_2 glycoprotein I. Factor B and C2 proteins of the second category, as well as C4 itself map to the major histocompatibility locus on chromosome 6 (16), while the IL-2 receptor has been assigned to chromosome 10 (17).

The addition of DAF to the complement-regulatory gene family on the long arm of chromosome 1 further increases the significance of this locus to the complement system. Although DAF shares ligand-binding specificity and C3 convertase regulatory function with three members, CR1, factor H, and C4bp, of this group of proteins, it possesses features that distinguish it from the other proteins of this group (reviewed in 18). DAF has a wider tissue distribution, *O*-linked oligosaccharides account for >40% of its M_r, and (cell-associated) DAF is anchored in membranes via a posttranslationally attached glycolipid structure. DAF is deficient in cells of patients with paroxysmal nocturnal hemoglobinuria (PNH). Further investigation of the structure of this region including molecular mapping and the search for genes encoding other complement regulatory proteins should be informative.

Summary

Delay-accelerating factor (DAF) protects host cells from complement-mediated damage by regulating the activation of C3 convertases on host cell surfaces. Using a panel of hamster-human somatic cell hybrids, the *DAF* gene was mapped to human chromosome 1. In situ hybridization studies using human metaphase cells further localized the gene to bands 1q31-41, with the largest cluster of grains at 1q32. This establishes the close linkage of the *DAF* gene to genes for four other proteins (C3b/C4b receptor or complement receptor 1, C3d receptor or complement receptor 2, factor H, and C4-binding protein) that share 60-amino-acid homologous repeats as well as complement-regulatory or -receptor activity, thereby enlarging the complement-regulatory gene family on the long arm of human chromosome 1.

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