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Genetic mapping of eight SH3 domain genes on seven mouse chromosomes

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The process of screening cDNA expression libraries with phage-optimized peptide ligands, termed cloning of ligand targets (COLT), was recently used to isolate a series of SH3 domain-containing proteins (Sparks et al. 1996). Among the 18 SH3 domain-containing proteins identified, nine were previously unreported from mouse. The structures of seven of the proteins whose genes are mapped in this study are diagrammed in Fig. 1. The function of many of the mouse proteins is being actively pursued by many laboratories. Three of the proteins, SH3P4, SH3P8, and SH3P14, are highly related in structure and have been discovered to bind synaptojanin and dynamin, and to be involved in endocytosis (de Heuvel et al. 1997; Ringstad et al. 1997). This family of proteins, which has also been discovered in human (Giachino et al. 1997), has been termed endophilin 1, 2, and 3. SH3P8 was also identified as a gene fused to MLL in human acute myeloid leukemia (So et al. 1997) and as a protein that binds to the Gag protein of murine leukemia viruses in a yeast two-hybrid screen (W. Kim, T. Torrey, H. Morse, unpublished observations). SH3P9, renamed amphiphysin II because of its strong similarity to amphiphysin, has been shown to be a component of the endocytic machinery (Butler et al. 1997; Ramjaun et al. 1997) as well as the cytoskeleton, where it may regulate the c-*Abl* tyrosine kinase (Kadlec and Pendergast 1997). SH3P12 has been observed to interact with c-*Cbl* in cells where it may play a role in both signal transduction pathways and regulation of the cytoskeleton (Ribon et al. 1998).

To learn more about the genes for the seven novel mouse proteins, we set out to map their chromosomal locations. Two sets of multilocus genetic crosses were analyzed for inheritance of the mouse genes encoding the SH3 family genes: (NFS/N or C58/J × *M. m. musculus*) × *M. m. musculus* (NMM and CMM; Kozak et al. 1990) and (NFS/N x *M. spretus*) × *M. spretus* or C58/J (NSS and NSC; Adamson et al. 1991). Probes were prepared by polymerase chain reaction (PCR) from the nine cDNA clones, seven of which have been deemed full-length (Sparks et al. 1996), and used in blots of restriction enzyme digests of genomic DNA.

Inheritance patterns were described for eight polymorphic fragments identified by the seven different probes. Restriction fragments used to identify specific loci are given in Table 1. Seven loci were typed in the *M. spretus* crosses and six loci in the *M. m. musculus* crosses. Comparisons of the inheritance patterns of these fragments with those of over 1200 loci previously typed and mapped in these crosses showed that eight loci could be positioned on seven chromosomes. Two genes were mapped to Chromosome (Chr) 19, but they were separated by more than 20 cM. The recombination data used to derive the map locations are given in Table 2.

In all but one case, all restriction fragments identified by a single probe could be mapped to the same locus. The exception, probe SH3P13, identified loci on two mouse chromosomes, Chrs 2 and 7. The two corresponding loci, *Sh3d2c1* and *Sh3d2c2*, were identified in both sets of crosses, indicating that neither represented a species-specific pseudogene. Previous data have shown that the human homolog of this SH3 domain gene, originally termed SH3GL3, is located at 15q24 (Giachino et al. 1997). One of the mouse loci identified by this probe, *Sh3d2c2*, mapped to a region of Chr 7 homologous to 15q21-26, identifying it as the mouse homolog of human SH3GL3. The nature of the second locus, *Sh3d2c*, remains to be determined.

Human map locations have also been defined for *Sh3d2a* and *Sh3d2b*. The homolog of *Sh3d2a* (SH3GL2) was mapped to 9p22, which is consistent with its position on mouse Chr 4. We previously reported the map location for *Sh3d2b* on mouse Chr 17 (Torrey et al. 1998), consistent with the human location for SH3GL1 on 19p13.3. Finally, another SH3 domain gene, *Amph* (amphiphysin), maps to mouse Chr 13 and human 7p14-p13 (Jenkins et al. 1995).

None of the other genes have been mapped in human, although mouse map locations predict positions for SH3D4 and SH3D5 on human chromosomes 8p21-23 and 10q respectively. Since map locations for *Dbn1l* and *Sh3d3* place them in regions with homology to two or more human chromosomes, we attempted to make

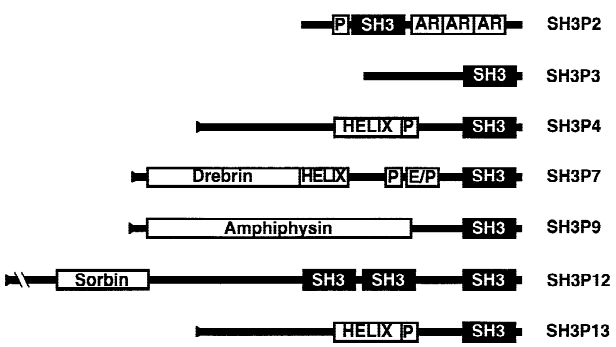

Fig. 1. Schematic diagram of the seven novel mouse proteins whose chromosome locations were mapped in this study. The proteins were identified in a COLT screen of a mouse 16-day embryo cDNA library (Sparks et al. 1996). Diagrams are to scale, with the SH3 domains (black boxes) representing approximately 60 amino acids. AR = ankyrin repeats; E/P = glutamate/proline-rich segments; HELIX = putative □-helical segments; and P = proline-rich segments. Regions with a high degree of sequence identity to amphiphysin, drebrin, and sorbin are also noted with boxes. GenBank accession numbers of the proteins are U58888 (SH3P2), U58889 (SH3P3), U58886 (SH3P4), U58884 (SH3P7), U60884 (SH3P9), U58883 (SH3P12), and U58887 (SH3P13). All reading frames are full-length, except for SH3P2 and SH3P3.

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Table 1. Restriction fragments used to identify SH3 domain loci in two sets of multilocus crosses.

Locus	Name	Probe[a]	Chr	Enzyme	Fragment Size (Kb)[b] NFS/N	Fragment Size (Kb)[b] *M. spretus*	Crosses Typed[c]
Amph2	Amphiphysin 2	SH3P9	18	*Pst*I	4.3	4.6	NSS, NSC
Dbn1l	Debrin 1 like	SH3P7	11	*Sac*I	3.2, 3.0	4.4, 3.0	NSS, NSC
Sh3d2a	SH3 domain protein 2A	SH3P4	4	*Sst*I	6.7		NMM, CMM
				*Pvu*II	8.6	7.9	NSS, NSC
Sh3d2c1	SH3 domain protein 2C1	SH3P13	2	*Bgl*II	16.0		NMM, CMM
				*Pvu*II	10.1	6.5	NSS, NSC
Sh3d2c2	SH3 domain protein 2C2	SH3P13	7	*Bgl*II	11.2, 4.6		NMM, CMM
				*Pvu*II		5.7	NSC
Sh3d3	SH3 domain protein 3	SH3P2	19	*Sac*I	23.0, 6.2		NMM, CMM
				*Apa*I	9.2, 7.1	9.0, 5.8	NSS, NSC
Sh3d4	SH3 domain protein 4	SH3P3	14	*Bam*HI	18.8		NMM, CMM
Sh3d5	SH3 domain protein 5	SH3P12	19	*Sac*I	16.0		NMM, CMM
				*Apa*I	3.8	8.7	NSS, NSC

[a] Probes were prepared by PCR amplification with oligonucleotides flanking the site of insertion of the various cDNAs in the pEXlox plasmid. cDNA clones are described elsewhere (Sparks et al. 1996).
[b] Fragment sizes are given only for variant-sized fragments typed in progeny DNAs. Loci typed in the *M. m. musculus* crosses produced fragments of identical sizes in NFS/N and C58/J.
[c] Crosses: NMM, (NFS/N × *M. m. musculus*) × *M. m. Musculus*
CMM, (C58/J × *M. m. musculus*) × *M. m. musculus*
NSS, (NFS/N × *M. spretus*) × *M. spretus*
NSC, (NFS/N × *M. spretus*) × C58/J

Table 2. Recombination frequencies for the SH3 domain genes and flanking marker loci.

Chr	Locus 1[a]	Locus 2	No. recombinant/total *M. spretus* crosses	No. recombinant/total *M. m. musculus* crosses	Recombination distances ± SE[b]
2	*Il2ra*	*Cacnb2*	2/94	1/67	1.9 ± 1.1
	Cacnb2	*Sh3d2c1*	1/92	1/30	1.6 ± 1.1
	Sh3d2c1	*Cchn1a*	0/96	0/82	(1.7)
	Sh3d2c1	*Vav2*	0/85	5/113	2.5 ± 1.1
4	*D4Rp18*	*Tyrp1*	0/65	1/116	0.6 ± 0.6
	Tyrp1	*Sh3d2a*	0/74	4/41	3.5 ± 1.7
	Sh3d2a	*Ifna*	1/83	0/37	0.8 ± 0.8
7	*Agc*	*Sh3d2c2*	4/74	2/121	3.1 ± 1.2
	Sh3d2c2	*Tyr*	3/70	5/130	4.0 ± 1.4
11	*Gk*	*Dbn1l*	0/58		(5.0)
	Dbn1l	*Gabra1*	16/80		20.0 ± 4.5
	Gabra1	*Olfr10*	2/80		2.5 ± 1.7
14	*Blk*	*Sh3d4*		5/59	8.5 ± 3.6
	Sh3d4	*sys*		0/100	(3.0)
	Sys	*Htr2a*		1/98	1.0 ± 1.0
18	*Ttr*	*Amph2*	12/98		12.2 ± 3.3
	Amph2	*Camk4,Syt4*	0/107		(2.8)
	Amph2	*Hsp74*	1/107		0.9 ± 0.9
	Hsp74	*Lox*	12/106		11.3 ± 3.1
19	*Cd5*	*Sh3d3*	6/66	7/52	11.0 ± 2.9
	Sh3d3	*Gin1*	14/62	7/58	17.5 ± 3.5
	Gin1	*Sh3d5*	5/80	2/72	4.6 ± 1.7
	Sh3d5	*D19Mit17*	4/53	1/56	4.6 ± 2.0
	D19Mit17	*Nrap*	6/59	6/64	9.8 ± 2.7

[a] Blot transfer methods and hybridization probes have been described previously for the marker loci used here (Kozak and Buckler 1997). Newly mapped genes are underlined.
[b] Recombination distances were calculated according to Green (1981). Loci were ordered by minimizing the number of recombinants. No double recombinants were identified within these intervals. When no recombinants were identified, distances are given in parentheses and represent the upper level of the 95% confidence interval. Typing data were stored and analyzed with the program LOCUS developed by C.E. Buckler (NIAID, Bethesda, MD).

chromosome assignments for these genes by Southern blot analysis of a human × rodent somatic cell hybrid panel obtained from BIOS (New Haven, Conn.; Carlock et al. 1986). The panel consists of 19 human × hamster hybrids and one human × mouse hybrid. The *Sh3d3* probe did not produce a clear human signal, but *Dbn1l* could be assigned to human Chr 7 and, based on its mouse location, is likely to map to 7p11-p13.

Our results indicate that these SH3 domain-containing proteins have a wide chromosomal distribution in mouse and human. Several of these genes show sequence homologies outside the SH3 domain, namely, the two amphiphysin genes and the small family consisting of SH3P4 (*Sh3d2a*), SH3P8 (*Sh3d2b*), and SH3P13 (*Sh3d2c2*) (Sparks et al. 1996; Giachino et al. 1997). However, these related genes are neither clustered nor map to chromosomes with known paralogous relationships as might be expected for families of genes derived by duplication and divergence. The remaining genes mapped in this study show little relatedness outside the SH3 domain, and, therefore, it is not surprising that they do not have clustered or paralogous map locations.

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