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Synaptotagmin genes on mouse Chromosomes 1, 7, and 10 and human Chromosome 19

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The synaptotagmins (p65s) are abundant secretory vesicle membrane proteins with calcium and phospholipid binding domains that may function in calcium-dependent exocytosis in neurons and some endocrine cells. Mutations of synaptotagmin in *C. elegans* and *Drosophila* result in weakness and uncoordinated movement and are lethal (Nonet et al. 1993; Di Antonio et al. 1993). cDNAs encoding three isoforms of rat synaptotagmin have been cloned (Perin et al. 1990, 1991; Geppert et al. 1991; Mizuta et al. 1994). There is 40% overall amino acid sequence identity among the three proteins, concentrated in a region that is highly homologous to the C_2 regulatory domain of protein kinase C. The synaptotagmin genes are differentially expressed in various brain regions. *Syt3* is also expressed in pituitary, thyroid, pancreatic islets, and several hormone-secreting cell lines (Mizuta et al. 1994). To evaluate the synaptotagmins as candidate genes for mouse neurological mutants, we mapped the three mouse synaptotagmin genes, using the rat cDNAs and two interspecific backcrosses.

Restriction fragment length polymorphisms for *Syt1, Syt2,* and *Syt3* were identified by digestion of genomic DNA from strains CAST/Ei, SPRET/Ei, and C57BL/6J (The Jackson Laboratory, Bar Harbor, Me.) and hybridization with cDNA probes (Table 1). The rat cDNA probes for *Syt1* (Perin et al. 1990), *Syt2* (Geppert et al. 1991; Perin et al. 1990), and *Syt3* (Mizuta et al. 1994) were previously described. Primers for the Mit microsatellite markers (Dietrich et al. 1992) were obtained from Research Genetics (Birmingham, Ala.).

Synaptotagmin 1 was previously localized to human Chromosome (Chr) 12 cen-q21 (Perin et al. 1991), a region that includes two conserved groups on mouse Chrs 10 and 15. No linkage was observed between *Syt1* and markers for mouse Chr 15. However, analysis of markers from Chr 10 demonstrated linkage (Fig. 1A). The observed gene order and distances on distal region of mouse Chr 10 were: centromere–*D10Mit10*–10.7 ± 5.8–*Syt1*–10.7 ± 5.8–*D10Mit136*–7.1 ± 4.9–*D10Mit14*. *Syt1* is thus located within the large conserved linkage group on human Chr 12 and mouse Chr 10 that includes the region between phenylalanine hydroxylase and *Erbb3* (Taylor et al. 1993). Recent mapping of two neurological mutants in this region, grizzled and jittery, relative to molecular markers (Kapfhamer and Burmeister, 1994) indicates that *Syt1* is located distal to both.

On the basis of the reported linkage of *Syt2* to the renin gene in the rat, we tested linkage between *Syt2* and markers near *Ren1* on mouse Chr 1 (Fig. 1B). The following gene order and distances were observed: centromere–*D1Mit5*–19.2 ± 7.7–*D1Mit140*–11.5 ± 6.3–*Syt2*–15.4 ± 5.0–*D1Mit37*. *Syt2* thus maps to a conserved linkage group on mouse Chr 1 and human Chr 1q that includes 39 mapped genes (Seldin et al. 1993).

Since no mapping data were available for *Syt3,* we probed The Jackson Laboratory BSS backcross which has been typed for markers that span the mouse genome (Rowe et al. 1994). Linkage was observed with markers from the proximal region of mouse Chr 7 (Fig. 1C), with the following gene order and distances: centromere–*D7Mit20*–4.3 ± 2.1–*D7Bir7*–9.6 ± 3.0–*Syt3, Kal*–6.4 ± 2.5–*Ldh3*. No recombinants (0/94) were observed between *Syt3* and the kallikrein gene family (*Kal*). To map human SYT3, we hybridized a Southern blot containing genomic DNA from the NIGMS Mapping Panel 2 (Coriell Institute, Camden, N.J.). A 17-kb hybridizing *Hin*dIII fragment was observed in human DNA and in cell line GM/NA 10449 with Chr 19 as its only human chromosome. None of the other single chromosome hybrid lines contained the human gene (data not shown). This result suggests that *Syt3* is located in the conserved linkage group on proximal mouse Chr 7 and human Chr 19q that is known to include at least 28 genes (Holdener et al. 1993).

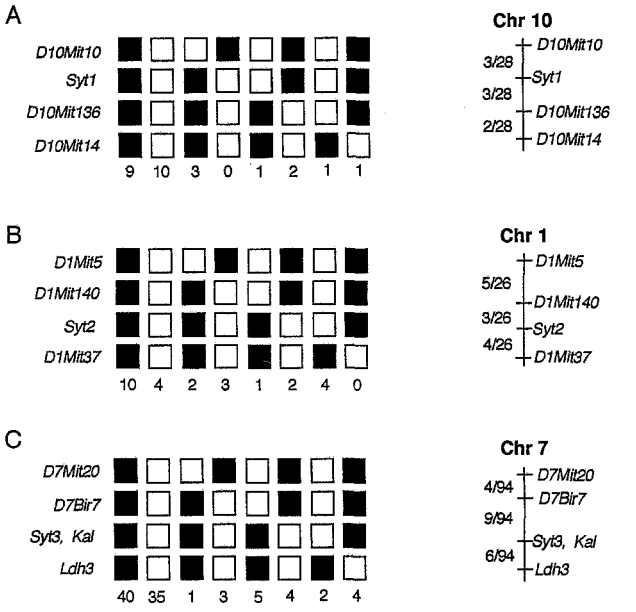

Fig. 1. Haplotypes and derived linkage maps for three mouse synaptotagmin genes. The interspecific backcross [(C57BL/6J-*med*^{tg1} × CAST/Ei)F_1 × C57BL/6J] (Kohrman et al. 1995) was typed for *Syt1* and *Syt2* by Southern blotting as previously described (Barrow et al. 1994). The Jackson Laboratory interspecific BSS backcross [(C57BL/6J × SPRET/Ei) × SPRET/Ei] (Rowe et al. 1994) was typed for *Syt3*. Each column represents one observed haplotype from the backcross mapping panel. The number of mice with each haplotype is indicated at the bottom of each column. Solid symbols, C57BL/6J alleles; open symbols, CAST/Ei or SPRET/Ei alleles. Derived maps of the relevant chromosome regions are shown at the right. MED accession #CREX 241, 242, 243.

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Table 1. Strain variation in *Taq* I fragments hybridizing with synaptotagmin cDNAs. The *Syt1* probe was the 1.7-kb *EcoRI/Xbal* cDNA fragment from clone pCMV65-5, and the *Syt2* probe was the 2.7-kb *Xbal* cDNA fragment from clone pCMV65-8 (Geppert et al. 1991 and T. Sudhof, personal communication). The *Syt3* probe was a 1.8-kb *PmacI/NarI* cDNA fragment (Mizuta et al. 1994). Restriction fragment lengths are given in kb. The fragments which were scored in segregating crosses are indicated in bold print.

LOCUS	C57BL/6J	CAST/Ei	SPRET/Ei
Syt1	7.2, 5.8, **4.3,** 2.7, 1.7, <1.0	**14.5,** 7.2, 5.8, **3.8,** 2.7, 1.7, <1.0	
Syt2	**4.3,** 1.7, 1.5, <0.5	4.5, **2.1,** 1.5, <0.5	
Syt3	**7.9**	7.9	5.0, 3.0

Syt3 is located near two neurological mutations, lumbosacral neuroaxonal dystrophy (*lnd*) causing dystrophic axons and progressive spastic paresis (Bronson et al. 1992), and quivering (*qv*), characterized by hindlimb paralysis, deafness of central origin, and male sterility (Green 1989; Holdener et al. 1993). To test for disruption of *Syt3* in quivering mutants, we probed Southern blots containing genomic DNA from C57BL6/J, C3H/HeJ, and homozygotes for three spontaneous mutant alleles: *qv/qv*, qv^{2J}/qv^{2J}, and q^{3J}/q^{3J}. Four hybridizing fragments were detected after digestion with *Sac*I (4.7, 3.8, 3.1, and 2.6 kb) and *Hin*dIII (15, 5.8, 2.4, and 1.4 kb). No differences in length or relative intensity of hybridizing fragments were observed in the three quivering alleles. Although we did not detect any altered fragments, more subtle mutations would not be detected by this method. *Syt3* remains an interesting candidate gene for these two neurological mutants.

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