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Localization of human and mouse *N*-ethylmaleimide-sensitive factor (NSF) gene: a two-domain member of the AAA family that is involved in membrane fusion

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The process of vesicle targeting and fusion in the secretory and endocytic pathways has been described by the SNARE hypothesis (Rothman 1994). This proposes that vesicles dock with specific target membranes by binding to membrane-specific SNAREs (soluble N-ethylmaleimide-sensitive factors attachment protein receptors); targeting specificity is also affected by the Rabs, a group of small soluble GTPases (Simons and Zerial 1993; Fischer von Mollard et al. 1994). After the vesicle has bound to the target membrane, the SNARE multimer is joined by the soluble SNAP proteins and *N*-ethylmaleimide-sensitive factor (NSF); this large complex is thought to allow membrane fusion, and the ATPase activity of NSF appears to be essential for this process (Rothman 1994). While there are a variety of different SNARES, Rabs, and SNAPs involved in membrane fusion, there is only one NSF, and the SNARE hypothesis describes NSF-dependent fusion.

Mammalian *N*-ethylmaleimide-sensitive protein was first identified as the protein that restored the ability of Golgi membranes that had been inactivated with the reagent *N*-ethylmaleimide, to re-engage in vesicular transport (Glick and Rothman 1987). *NSF* was subsequently cloned from Chinese hamster cells by Wilson and colleagues (Block et al. 1988; Wilson et al. 1989). *NSF* is a member of the AAA (*A*TPases *A*ssociated with diverse cellular *A*ctivities) gene family. The genes are most related throughout an approximately 200-amino acid domain (the AAA domain) that binds ATP; however, the family is notable not only for its conservation but also for the diverse functions of its proteins in eukaryotic cells. The family can be subdivided into those with either one or two ATP-binding domains. NSF is a two-domain member of the AAA family. VCP, the valosin-containing protein that is also involved in membrane fusion, is another two-AAA domain protein (Schmidt et al. 1985; Pleasure et al. 1993), as is PAS1, a *Saccharomyces cerevisiae* protein required for peroxisome biogenesis (Erdmann et al. 1991). Mammalian one-domain proteins include P26S4, subunit 4 of the 26S protease (Dubiel et al. 1992; Hoyle and Fisher 1996); MSS1 (S7) subunit 7 of the 26S protease (Shibuya et al. 1992); TBP1, the HIV Tat binding protein 1 (Nelbock et al. 1990); TRIP1, the thyroid-hormone-receptor interacting protein (Lee et al. 1995). The AAA family is highly conserved between eukaryotes, and members are known in archaebacteria and eubacteria (Hershko and Ciechanover 1992; Confalonieri et al. 1994; Tomoyasu et al. 1995).

A partial human *NSF* sequence has been described by Hong and colleagues (unpublished, accession no. U03985) that includes a portion of the 5′ and 3′ untranslated regions (UTR) and the complete coding region. However, from database searches performed with the Blast program, we determined that this human *NSF* partial cDNA sequence has identity to a 415-bp EST (Accession no. R61048). The first nucleotide of the EST corresponds to bp 2145 of the partial cDNA sequence; the EST overlaps the human *NSF* coding region and includes 3′ UTR sequence (Fig. 1). The 3′ UTR preceding the likely polyadenylation site in the EST has 67% identity to the corresponding region of the mouse *Nsf* 3′ UTR. Therefore to map human *NSF,* we designed primers to the *NSF* 3′ UTR, based on the EST sequence (Fig. 1). A 114-bp 3′ UTR product was amplified in a human monochromosomal somatic cell hybrid mapping panel (described in Hernandez et al. 1994). The amplification was human specific. Only two lanes gave amplification products: total human and cell line PCTBA1.8, which contains human Chr 17 only on a mouse background. This amplification result indicates that the *NSF* gene maps to Chr 17.

To determine a regional mapping position for NSF and to confirm our cell hybrid panel results, we isolated *NSF*-containing human cosmids for FISH mapping. We designed PCR primers to the *NSF* coding region (Fig. 1) and amplified the expected 856-bp fragment from a human cerebellum cDNA library (Stratagene). This product was subcloned and a recombinant, pBC383, was sequenced (as described in Yulug et al. 1995) to confirm identity to the human *NSF* cDNA sequence. The 856-bp *NSF* product was hybridized to a Chr 17-enriched gridded cosmid filter, as described by Yulug and associates (1994). The 856-bp fragment was hybridized to *Eco*RI digests of positive cosmids, and the following fragments were detected: in two cosmids ICRFc105G06141, ICRFc105F05161—4.5 kb and 7 kb; in ICRFc105E1036—4.0 kb and 7 kb; in ICRFc105E06181—7 kb; in ICRFc105G0152—6 kb. ICRFc105G06141 and ICRFc105F05161 were chosen for FISH mapping, as described by Yulug and colleagues (1994). Our FISH mapping results show that both cosmids hybridize to human Chr 17q21-q22 (data not shown). We confirmed that these two different cosmids contained *NSF* sequences by vectorette PCR sequencing adapted from Riley and coworkers (1990). Our strategy involved the generation of an *Hae*III vectorette library from cosmid DNA followed by PCR with primer NSFCodR (Fig. 1) and a vectorette primer. The 200-bp product obtained was sequenced and corresponds to exonic sequence extending from bp 1493 to 1643 of file U03985.

To map *Nsf* in the mouse genome, we screened a Balb/c neonatal brain cDNA library (Stratagene) with the 856-bp human coding region PCR product; a 3.5-kb mouse *Nsf* cDNA was isolated, as confirmed by partial sequencing. We amplified a 156-bp 3′ UTR fragment from this cDNA (Fig. 1). The 156-bp fragment was hybridized to *Bam*H1 digests of DNAs from the European Interspecific Backcross (EUCIB) as described in Hoyle and Fisher (1996). EUCIB consists of a backcross of C57BL/6 females crossed to *Mus spretus* males. A range of highly variant markers

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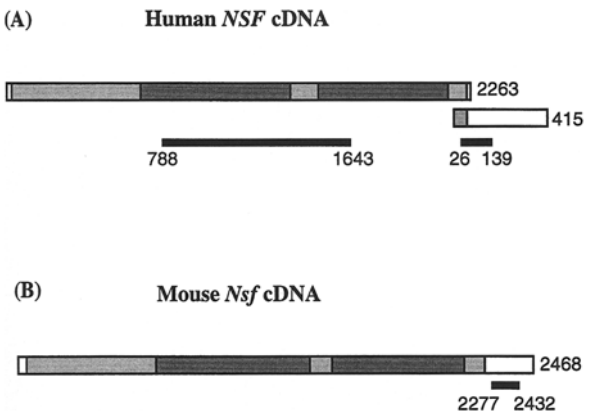

Fig. 1. Human and mouse *NSF* cDNAs. Coding region is shown by the lightly stippled box; the two AAA domains are shown by the darkly stippled box and are positioned according to K.-U. Fröhlich; PCR products are marked by the thick lines. **(A)** Human *NSF* partial cDNA (U03985), which is 2263 bp, with coding region from bp 22 to 2253; ATPase domains extend from p 649 to 1389 and 1501 to 2133. This sequence has identity, beginning at bp 2145, to a 415-bp EST (R61048) which is shown overlapping the larger cDNA. To map *NSF* in a somatic cell hybrid panel, a 114-bp product was amplified from the 3′ UTR, with primers to bp 26 to 46 (NSFUtrF TGAAACACACAGTGACCAAGG) and bp 139 to 121 (NSFUtrR GAGCACATGTTGAAGGTAG) of the EST. To create a hybridization probe for isolating *NSF* cosmids, we amplified an 856-bp fragment, using primers extending from bp 788 to 807 (NSFCodF TCCTGTTATATGGACCCCCA) and bp 1643–1625 (NSFCodR AGAAGCACGCTGACCAATG) of the cDNA. **(B)** Subsequent to our isolation of the mouse cDNA, *Nsf* was cloned by Périer and colleagues (gene named: *SKD2*, Accession no. U10120; Périer et al. 1994) in a complementation analysis of mouse cDNAs that would correct a potassium transport growth defect in *S. cerevisiae*. The following figures and diagram refer to this cDNA, which is 2468 bp, with coding region from bp 34 to 2268; ATPase domains extend from bp 661 to 1401 and 1513 to 2145. To map *Nsf* in the mouse, a 156-bp 3′ UTR probe was amplified from cDNA with primers to bp 2277 to 2298 (NsfF TGTCTACAACAGTGACCAAGGG) and to bp 2412 to 2432 (NsfR TCTCAAAAGCACGAGGGAGT).

has been localized within almost 1000 backcross progeny, providing anchor loci for the genetic assignment of unmapped probes (for example, see Hernandez et al. 1994; Yulug et al. 1994; Hoyle and Fisher 1996). The 156-bp *Nsf* probe detects only one *Bam*H1 fragment of 3.0 kb in C57BL/6 DNA; the probe detects only one *Bam*H1 fragment of 8.0 kb in *M. spretus* DNA. The segregation of the restriction fragment length variants was scored in the backcross of F_1 progeny with *M. spretus*, and in the backcross of F_1 progeny to inbred C57BL/6 animals. In our hybridizations to the EUCIB backcross DNAs, we detected no extra fragments in any of our DNAs and no independent segregation of any of the observed fragments; therefore, we appear to be detecting the *Nsf* locus only.

An analysis of the segregation results was performed with the *MBx* linkage program (version 2.0), which placed *Nsf* close to the locus *D11Mit10*, which maps approximately 63 cM from the centromere (Fig. 2). Haplotype analysis of the results places the locus probably proximal of *D11Mit10*, in a region that contains no obvious mouse mutations for which *Nsf* might be a candidate. We have an excellent map of homologous regions between human Chr 17 and mouse Chr 11, and the localization in mouse accords well with the human localization, on the current comparative map (Mouse Genome Database: http://www.informatics.jax.org/).

We have added an extra marker to the human Chr 17–mouse Chr 11 homologous linkage group and mapped an important component of the membrane fusion pathway. We note that disinhibition-dementia-Parkinsonism-amyotrophy complex maps to 17q21-q22 (Wilhelmsen et al. 1994), as does a possibly allelic disorder, rapidly progressive Parkinsonism and dementia with pallidoponto-nigral degeneration (Wijker et al. 1996). We suggest that it

(A)

Marker	No. of Recombinants	cM
D11Nds19	19/60	32±6
D11Mit31	14/60	23±5
D11Mit36	12/60	20±5
D11Mit10	3/60	5±3
D11Mit11	11/56	20±5

(B)

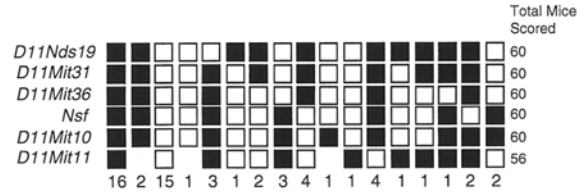

Fig. 2. Mapping *Nsf* in the mouse. The 156-bp mouse 3′ UTR product detected one 3.0-kb C57BL/6 fragment and one 8.0-kb *M. spretus* fragment in *Bam*HI digests of EUCIB DNAs. Access to the full EUCIB Chr 11 mapping panel is available through the Internet at http://www/hgmp.mrc.ac.uk/MBx/MBxHomepage.html. **(A)** Analysis of the segregation of these fragments (data freely available through EUCIB) showed *Nsf* maps to mouse Chr 11. The table shows the number of recombinants between *Nsf* and the marker indicated. **(B)** Haplotype analysis indicates *Nsf* is proximal of the marker *D11Mit10*. Each column represents a chromosome that was identified in the progeny. A black box represents a homozygous mouse, a white box represents a heterozygous mouse. The number of offspring inheriting each haplotype is listed at the bottom of each column. The total number of mice scored for each locus is listed on the right-hand side. A blank space means not determined.

would be worth examining the *NSF* DNA sequence in both sets of patients, because mutant *NSF* may give rise to a neurological phenotype. *NSF* is preferentially expressed in the mammalian nervous system (Püschel et al. 1994), and an aberrant *Drosophila Nsf* gene results in defective synaptic transmission (Pallanck et al. 1995).

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