SHORT COMMUNICATION

Mapping of the Gene (NRAP) Encoding N-RAP in the Mouse and Human Genomes

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N-RAP is a nebulin-related actin-binding protein found at the myotendon junction in skeletal muscle and at the intercalated disks in cardiac muscle. We mapped the NRAP gene to mouse chromosome 19 using interspecific crosses and to human chromosome 10 using radiation hybrid panels. Comparative analysis of the mouse and human genomes indicates that the NRAP gene is located in regions of conserved synteny between the two species.

N-RAP is a recently discovered protein that is expressed exclusively in striated muscle (9). The C-terminal half of N-RAP contains an actin-binding domain with sequence homology to nebulin, while a LIM domain is found at its N-terminus. It is localized at the myotendon junctions in skeletal muscle and at the intercalated disks in cardiac muscle. N-RAP is hypothesized to perform an anchoring function, linking the terminal actin filaments of myofibrils to protein complexes located beneath the sarcolemma (9). The name of this protein stands for nebulin-related anchoring protein, and it was chosen to reflect both the means of its discovery as a nebulin-related protein and its proposed function as an anchoring protein for actin filaments. Here we report the mapping of the gene encoding N-RAP in the mouse genome using interspecific crosses (2), as well as in the human genome using radiation hybrids (13).

Nrap was mapped in mice by analysis of the progeny of two sets of multilocus crosses: (NFS/N or C58/J × *Mus musculus musculus*) × *M. m. musculus* (8) and (NFS/N × *Mus spretus*) × *M. spretus* or C58/J (1). Progeny of these crosses have been typed for over 1000 markers distributed over the 19 autosomes and X chromosome including the chromosome 19 markers *Gin1* (Gross virus integration site), *D19Mit17* (chromosome 19 marker, MIT-17), and *Mif-ps9* (macrophage migration inhibitory factor-related sequence 9). *Gin1* and *Mif-ps9* were typed as described (7). *D19Mit17* was typed using primers and assay conditions described by Deitrich and his colleagues (3, 4). *Nrap* was typed using purified insert from clone SC-1, which contains nucleotides 3534 to 4588 of the full-length mouse N-RAP cDNA (GenBank Accession No. U76618) (9). Recombination was determined according to Green (5), and loci were ordered by minimizing the number of recombinants.

The probe for *Nrap* identified *Eco*RI fragments of 12.7 kb in *M. spretus* and 17.4 kb in NFS/N and C58/J. *Hpa*I digestion produced fragments of 19.4 kb in *M. m. musculus* and 12.7 and 8.0 kb in NFS/N and C58/

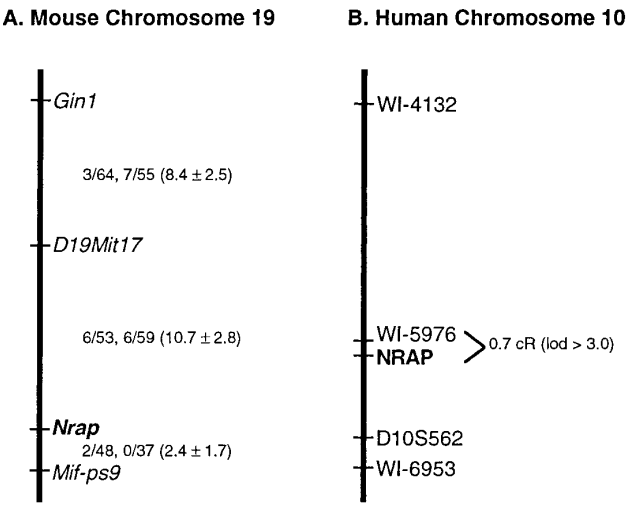

FIG. 1. Genetic map location of the gene encoding N-RAP on mouse chromosome 19 (**A**) and on human chromosome 10 (**B**). Recombination fractions for adjacent loci are given to the right of the mouse map, with the first fraction representing data from the *M. m. musculus* cross and the second from the *M. spretus* crosses. The recombinational distances calculated from the combined data are given in parentheses (mean ± standard error). The human map shows the location of NRAP relative to several radiation hybrid markers in the region (6).

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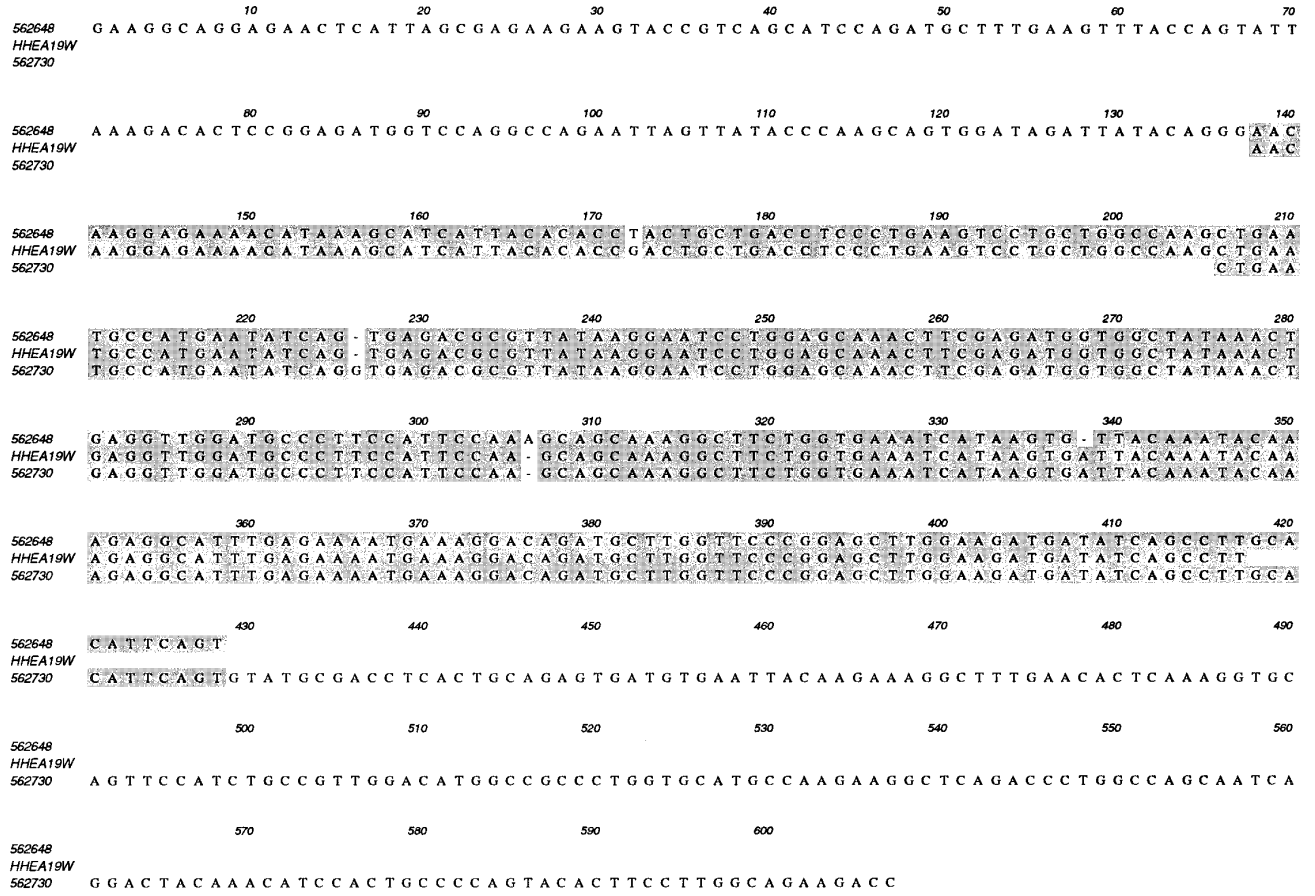
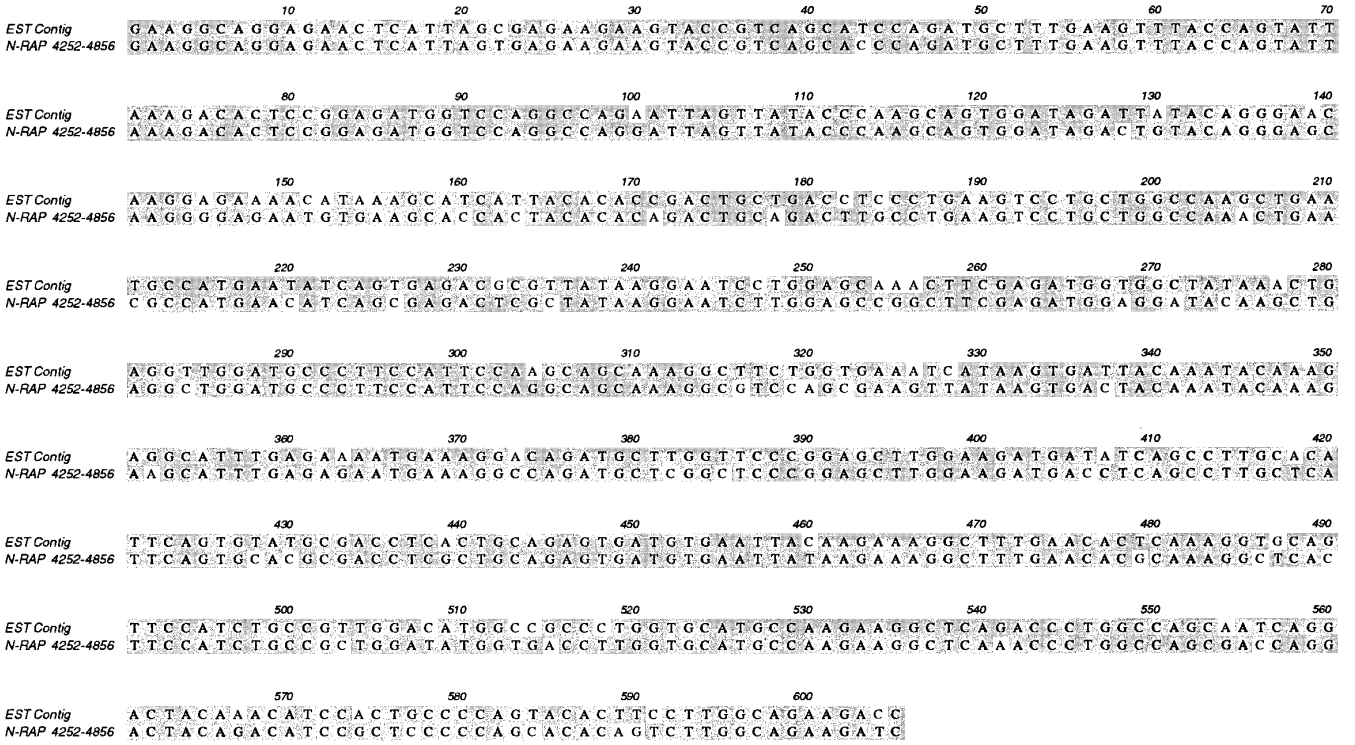
A. Alignment of Human ESTs



B. Alignment of Human EST Contig with Mouse N-RAP



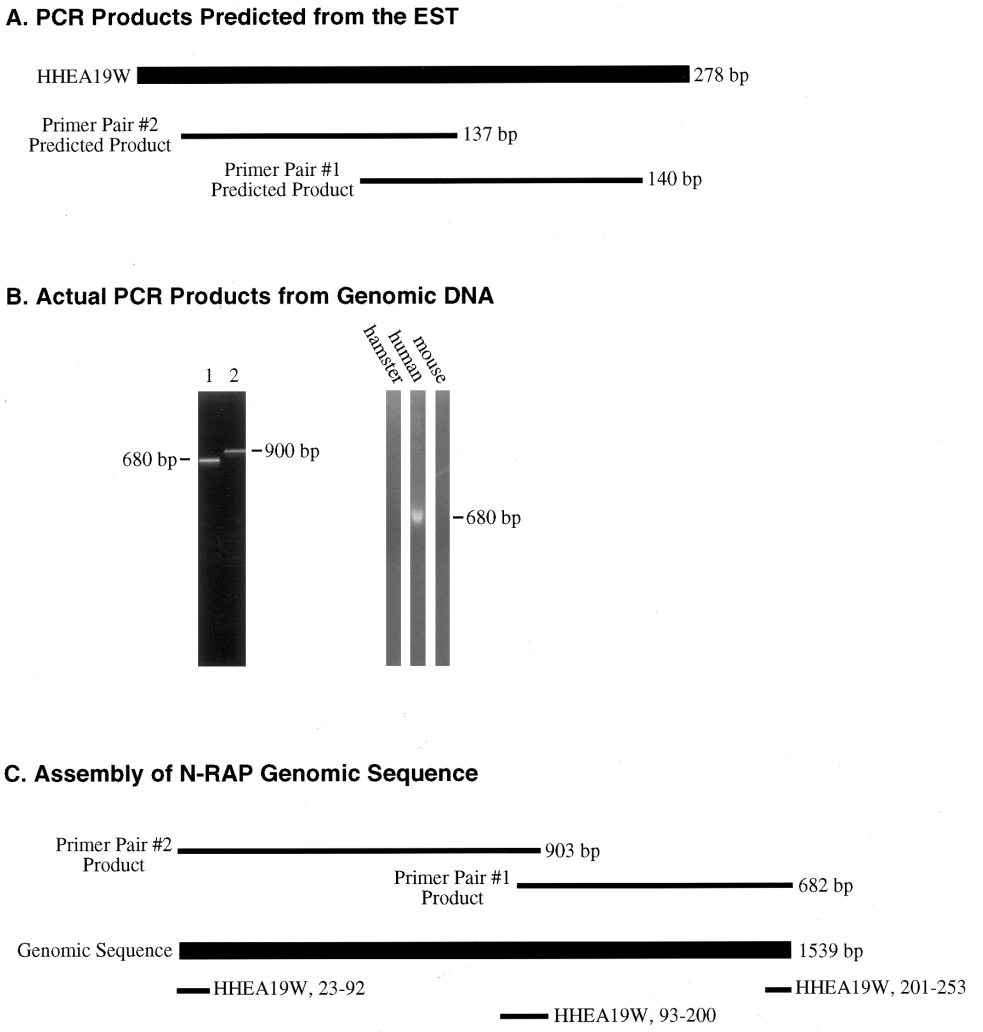


FIG. 3. Testing PCR primers for radiation hybrid mapping. (**A**) The schematic alignment of predicted PCR products from a region within cDNA clone HHEA19W is shown. Primer pair 1 consisted of 5′-AGCAAACTTCGAGATGGTGGC-3′ (forward primer) and 5′-CGGGAACCAAGCATCTGTCC-3′ (reverse primer); Primer pair 2 consisted of 5′-TCATTACACACCGACTGCTGACC-3′ (forward primer) and 5′-GAAGGGCATCCAACCTCAGTTTA-3′ (reverse primer). (**B**) The left-hand panel shows the actual PCR products from human genomic DNA using the primer pairs described in (**A**). Note that the products are larger than expected from the cDNA, suggesting the presence of introns. The right-hand panel shows the species specificity of the primer pair used for radiation hybrid mapping. The forward primer was 5′-CAAACTTCGAGATGGTGGCTATAAA-3′, and the reverse primer was the same as for primer pair 1 in (**A**). No product is amplified from the rodent genomic background that is present in the hybrid cells. PCR conditions were as previously described (11). (**C**) Schematic assembly of the sequence obtained from the PCR products shown above (B, left) to yield 1539 basepairs of human NRAP genomic sequence (GenBank Accession No. U96486). The correspondence to cDNA sequence from the cardiac EST is shown at the bottom. The results confirm that the PCR products are derived from the human NRAP gene. Double-stranded nucleotide sequence was obtained by cycle sequencing using either the AmpliCycle Sequencing Kit or the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (both from Perkin–Elmer, Foster City, CA), according to the manufacturer's instructions.

J. Inheritance of the variant fragments was compared with inheritance of over 1000 markers previously typed in these crosses, and linkage was observed with markers on chromosome 19. As indicated in Fig. 1A, *Nrap* mapped between *D19Mit17* and *Mif-ps9*.

To obtain sequence information concerning the human NRAP gene, we compared the mouse N-RAP cDNA sequence against the GenBank database of expressed sequence tags (ESTs). The best matches include unpublished sequence from a human cardiac EST, clone HEA19W (GenBank Accession No. Z36252), as well as from two human skeletal muscle ESTs (Gen-

FIG. 2. (**A**) Alignment of three overlapping human ESTs with high homology to mouse N-RAP. The ESTs are clone HHEA19W from human cardiac muscle (GenBank Accession No. Z36252) and clones 562648 and 562730 from human skeletal muscle (GenBank Accession Nos. AA102195 and AA111837, respectively). (**B**) Alignment of the contiguous sequence derived from the human ESTs with mouse NRAP. The human EST contig is 88% identical to the mouse skeletal muscle NRAP cDNA between basepairs 4252 and 4856 (GenBank Accession No. U76618).

Bank Accession Nos. AA102195 and AA111837). These three ESTs exhibit considerable overlap and align to form 605 bp of contiguous sequence (Fig. 2A). The EST contig aligns with basepairs 4252 to 4856 of the full-length mouse N-RAP cDNA (Fig. 2B). We used the sequence alignment shown in Fig. 2B to design a series of PCR primers that would amplify regions of the NRAP gene from human genomic DNA, but would not amplify homologous products from rodent DNA (Fig. 3A). These PCR primers consistently yielded products from human DNA that were larger than predicted (Fig. 3B, left), but did not amplify products from mouse or hamster DNA (Fig. 3B, right). Double-stranded sequence data from the PCR products showed a perfect match to the HEA19W sequence, but with the addition of two introns (Fig. 3C). Additional experiments using 23 different primer pairs covering the available sequence from clone HHEA19W consistently yielded PCR products with sizes consistent with the gene structure shown in Fig. 3C (data not shown). The results demonstrate that the primers specifically amplify a region of the human N-RAP gene, but do not amplify N-RAP from the hamster genome, which was present in the recipient cells used in radiation hybrid mapping.

Mapping of NRAP in the human genome was performed by PCR analysis using the GeneBridge 4 radiation hybrid panel (13). Statistical analysis of the data was performed using the RHMAPPER software package (software by D. Slonim, L. Stein, L. Kruglyak, and E. Lander). As shown in Fig. 1B, NRAP maps to human chromosome 10 between the radiation hybrid markers D10S562 and WI-5976 (LOD $>$ 3.0). This region corresponds to cytogenetic bands 10q24–q26 (12).

Comparative analysis indicates that the NRAP gene is located in a region of conserved synteny between human and mouse chromosomes. The mouse *Nrap* gene is thus within a region of chromosome 19 encompassing 30 cM that includes many genes that have homologs located on human chromosome region 10q23–q26 (10). The assignment of NRAP confirms the previously demonstrated homology between these regions.

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