BRIEF MAPPING REPORTS

Chromosomal Mapping of the Tankyrase Gene in Human and Mouse

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Functional gene description: Tankyrase (TNKS) is a novel gene with homology to both ankyrins and the catalytic domain of poly(ADP-ribose) polymerase (PARP) that was recently cloned using a yeast two-hybrid screen with the telomere-specific DNA binding protein TRF1 (1). Tankyrase protein is located at telomeres, centrosomes, and nuclear pore complexes (1; S. Smith and T. de Lange, in preparation). *In vitro* studies have demonstrated that tankyrase has PARP activity and that both tankyrase and TRF1 can function as acceptors for ADP ribosylation. Since ADP ribosylation of TRF1 decreases its telomeric DNA binding activity *in vitro,* the TNKS gene may function by modulating telomere length.

Description of clone or DNA: For human TNKS mapping, a PCR specific for the TNKS gene was utilized. The primers (forward, 5′CGAAGTGCTAGGGGAGTCCG; reverse, 5′GTGGGAGAGGCTGGG GTGGT) amplified a 169-bp fragment from the TNKS gene (Accession No. AFO82556). For mouse Tnks mapping, a mouse EST (Accession No. AA415426) that had 98% sequence homology with human TNKS was used.

Source: The source of cDNA, as described above, was sequence from a human cDNA clone and a mouse cDNA clone.

Method used to validate gene identity: Gene identity was validated by specific amplification and, for the mouse EST, stringent hybridization to a single disparate-sized restriction fragment in the two species of mouse used in the mapping panel.

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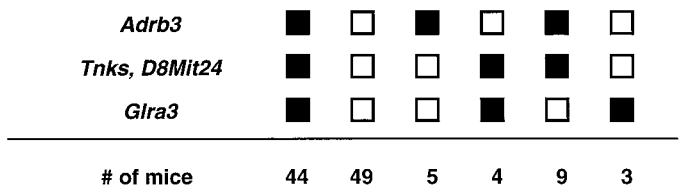

FIG. 1. Chromosomal localization of Tnks. The segregation of these genes among flanking genetic loci on mouse chromosome 8 in [(C3H/HeJ-gld × *Mus spretus*)F1 × C3H/HeJ-gld] interspecific backcross mice is shown. Filled boxes represent the homozygous C3H pattern and open boxes the F1 pattern. The number of mice with each haplotype is shown in each column. The mapping of the reference loci as well as the relationship to other mouse chromosomal markers is available on the Internet: use htpp://www.informatics.jax.org/crossdata.html to enter DNA Mapping Panel Data Sets from MGD, then select the Seldin cross and Chromosome. A consensus map position of 18 cM on mouse chromosome 8 is derived from interpolation using the most recent composite map (http://www.informatics.jax.org/bin/ccr/current/index).

Flanking markers used: The human location was defined relative to the large number of markers typed in the Stanford G3 radiation hybrid panel (http://www-shgc.stanford.edu/RH/index.html). The strongest linked marker was AFM193xh4. For the mouse, the mapping of the reference loci (Fig. 1) as well as the relationship to other mouse chromosomal markers is available on the Internet: use htpp://www.informatics.jax.org/crossdata.html to enter DNA Mapping Panel Data Sets from MGD, then select the Seldin cross and Chromosome.

Method of mapping: The human chromosomal location of TNKS was defined using the Stanford G3 radiation hybrid map. The mouse chromosomal location was defined using a panel of DNA samples from a well-characterized interspecific cross. Informative restriction fragments that segregated in the interspecific cross were identified on Southern blot hybridization using a cDNA probe specific for Tnks according to methods previously described (2): *Pst*I fragments (C3H/HeJ-gld, 1.3 kb; *Mus spretus,* 3.5 kb).

Results: For human, TNKS was positioned at 17.6 cR_{10000} on human chromosome 8 with a LOD of 8.2 on the G3 map. The 1000:1 confidence interval is 6–30 cM (Généthon position) of chromosome 8. For the mouse, comparison of the haplotype distribution of the Tnks-specific polymorphisms and those previously defined in this interspecific cross (Fig. 1) indicated the following relationships (±standard error): on mouse chromosome 8, centromere–Adrb3–7.9 ± 2.5 cM–Tnks, D8Mit24–10.5 ± 2.9 cM–Glra3. This corresponds at a consensus position of 18 cM on mouse chromosome 8 and helps define the homology relationship between regions of human and mouse chromosome 8 (see http:/www.ncbi.nlm.nih.gov/Homology/http://www.ncbi.nlm.nih.gov/genemap/).Interestingly TNKS/Tnks map to positions close to ankyrin 1 (ANK1/Ank1) located on hu-

man chromosome 8 (60.0–65.8 cM) and mouse chromosome 8 (9.5 cM). TNKS is more closely related to the ANK1 gene than to other ank repeat-containing genes; the ank repeat is 39% identical to the repeat domain in ANK1, suggesting a common derivation. It is notable that these results exclude Tnks as a candidate gene for the recently identified locus on distal mouse chromosome 2 that has been implicated in telomere regulation in the mouse (3).

Homologies: Homologies are as indicated above.

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Mapping of the Human NBC3 (SLC4A7) Gene to Chromosome 3p22

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Functional gene description: NBC3, designated SLC4A7, is a new member of the sodium bicarbonate cotransporter (NBC) family (6) cloned from a human muscle cDNA library (5). Muscle NBC3 (mNBC3) encodes a 1214-residue polypeptide with 12 putative membrane-spanning domains. mNBC3 is 78% homologous to NBC2 (3) and 39 and 46% homologous to pNBC1 and kNBC1, respectively (1, 2). The mNBC3 transcript is strongly expressed in skeletal muscle with less expression in heart. To begin to understand the genetic factors responsible for this pattern of expression, we have isolated the complete NBC3 gene (SLC4A7) and mapped it to chromosome 3p22.

Name of clone or DNA: A BAC human genomic DNA clone, 15, containing the entire human NBC3 gene was used for FISH analysis.

Description of clone: Primers designed for screening the BAC human library were 5′-CTGCAACAGTGTCAATAAGTCATG-3′ and 5′-ACTGAAGTCATGAACACAGAGAGGC-3′. The 160-bp PCR product was derived from exon 20.

Source: The BAC clone 15 was isolated using a PCR-based screen of a human genomic BAC DNA library (Genome Systems).

Method used to validate gene identity: A series of primers was designed based on the known mNBC3 cDNA sequence (5). These primers were used for cycle sequencing of the BAC clone 15. Sequences at the intron–exon boundaries of the NBC3 gene were determined by aligning the cDNA sequence with the genomic sequence.

Flanking markers: No flanking markers were used.

Method of mapping: The chromosomal localization of the NBC3 gene was determined by fluorescence *in situ* hybridization using as a probe the BAC genomic clone 15 containing this gene. DNA from clone 15 was identified by sequencing and was then labeled with digoxigenin–dUTP by nick-translation. Labeled probe was combined with sheared human DNA and hybridized to normal metaphase chromosomes derived from phytohemagglutinin-stimulated peripheral blood lymphocytes. Specific hybridization signals were detected by incubating the hybridized slides in fluoresceinated antidigoxigenin antibodies followed by counterstaining with DAPI for one-color experiments. Probe detection for two-color experiments was accomplished by incubating the slides in fluoresceinated antidigoxigenin antibodies and Texas red avidin followed by counterstaining with DAPI. A total of 80 metaphase cells were analyzed with 74 exhibiting specific labeling.

Results: The initial experiment resulted in specific labeling of the short arm of a group A chromosome, which was believed to be chromosome 3 on the basis of size, morphology, and banding pattern. A second experiment was conducted in which a biotin-labeled probe that is specific for the centromere of chromosome 3 was cohybridized with clone 15. This experiment resulted in specific labeling of the centromere and the arm of chromosome 3. Measurement of 10 specifically labeled chromosomes 3 demonstrated that NBC3 maps to a position that is 64% of the distance from the centromere to the telomere of chromosome arm 3p, an area that corresponds to band 3p22 (Fig. 1).

Additional comments: Recently, Olsen *et al.* (4) have studied a family with dilated cardiomyopathy in which the disease gene has been mapped to a 30-cM region of chromosome 3p22–3p25. The finding that NBC3 maps to this region suggests that it is a new candidate gene potentially causing familial dilated cardiomyopathy.

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