

Research article

Mapping of the Mouse Actin Capping Protein Beta Subunit Gene

Marilyn C. Hart², Yulia O. Korshunova³ and John A. Cooper¹

Address: ¹Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110, USA., ²Winona State University, Winona, MN 55987, USA. and ³Department of Genetics, Washington University, St. Louis, MO 63110, USA.
E-mail: ² mhart@VAX2.WINONA.MSUS.EDU ³ yuliak@sequencer.wustl.edu ¹ jcooper@cellbio.wustl.edu

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Abstract

Background.: Capping protein (CP), a heterodimer of α and β subunits, is found in all eukaryotes. CP binds to the barbed ends of actin filaments *in vitro* and controls actin assembly and cell motility *in vivo*. Vertebrates have three isoforms of CP β produced by alternatively splicing from one gene; lower organisms have one gene and one isoform.

Results.: We isolated genomic clones corresponding to the β subunit of mouse CP and identified its chromosomal location by interspecies backcross mapping.

Conclusions.: The CP β gene (*Cappb1*) mapped to Chromosome 4 between *Cdc42* and *D4Mit312*. Three mouse mutations, snubnose, curly tail, and cribriform degeneration, map in the vicinity of the β gene.

Background

Capping protein (CP) is a ubiquitous actin binding protein that regulates actin assembly and cell motility. CP is a heterodimer composed of α and β subunits, each approximately 30 kD. Lower organisms, including *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster*, have one gene and one isoform for each of the CP α and β subunits [1,2,3]. Vertebrates contain three α subunit isoforms encoded by three different genes and three β subunit isoforms (β_1 , β_2 , β_3) produced from one gene by alternative splicing [4,5,6].

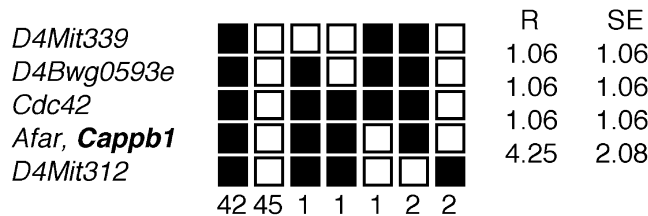
Results and Discussion

To confirm the genomic complexity of the β subunit of mouse CP, Southern blots of mouse genomic DNA were cleaved with several restriction enzymes and independently probed with mouse β_1 and β_2 cDNAs. The β_1 and β_2 probes each hybridized to one identical band. To obtain genomic clones that correspond to this pattern, a 129SV genomic library in the lambda FIXII vector (Stratagene) was screened using the complete β_1 cDNA as probe. Positive plaques were purified according to standard proce-

dures [7] and the phage DNA isolated using DEAE-cellulose [8]. Comparison of the hybridization pattern for the genomic clones with the mouse genomic DNA revealed that the patterns were identical, confirming the presence of a single β gene in the mouse genome.

In chicken, the β_1 and β_2 isoforms have been described as the differentially spliced products of a single gene, with the β_1 cDNA containing a 113 bp exon that is absent in the β_2 cDNA [4]. To determine if the murine β_2 isoform is spliced in a manner identical to that of chicken, the sequence of the β_2 cDNA was compared to the corresponding genomic region. Sequence comparison revealed that alternative splicing of the β_2 gene occurs in an identical manner in mice. The mouse intron is also 113 bp in length with the exon sequence and surrounding intron sequence containing the canonical splice donor (AGA) and acceptor (AGG) sites at either end of the intron. The human gene for CP β has a similar structure, based on the sequence of clone HS657E11, located at Chr1 p35.1-p36.23, from the Sanger Centre (www.sanger.ac.uk/HGP/Chr1/).

Jackson BSS Chromosome 4

**Figure 1**

Haplotype data for the mapping of the mouse CPβ gene showing a portion of Chromosome 4 with loci linked to *Cappb1*. Loci are listed in order with the most proximal at the top. The black boxes represent the C57BL/6J allele and the white boxes the SPRET/Ei allele. The number of animals with each haplotype is given at the bottom of each column of boxes. The percent recombination (R) between adjacent loci is listed to the right, with the standard error (SE) for each R. Missing typings were inferred from surrounding data where assignment was unambiguous.

To determine the chromosomal location of marine *Cappb1*, we identified interspecies variations of genomic sequences and mapped their chromosomal locations using an interspecies backcross panel from Jackson Laboratory [9]. The mapping panel is anchored to maps from crosses by various known genes, retroviral loci, and the *D#Mit* loci. The strain distribution pattern of each polymorphism in the interspecific backcross was determined and used to position the *Cappb1* gene on the map (Figure 1).

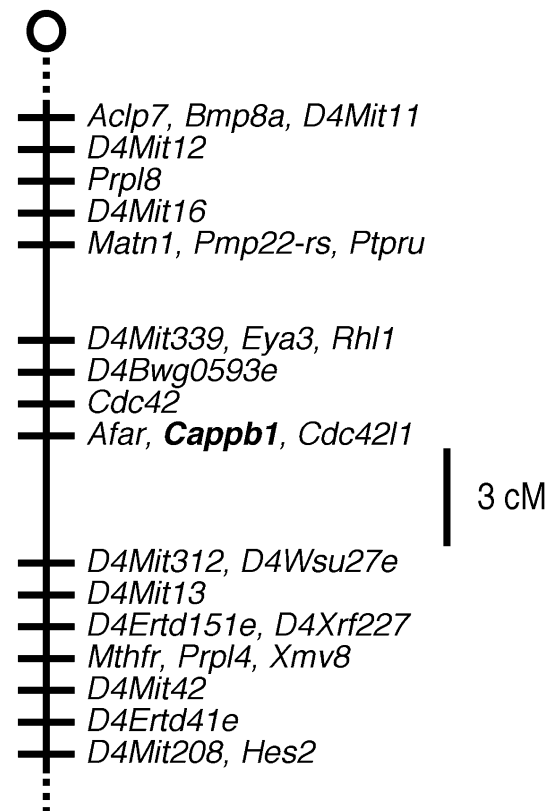
A region corresponding to the 3' UTR of the *Cappb1* gene was PCR-amplified using parental genomic DNA and gene-specific primers (5'TTTTCCCTCTTCTTTCC3' and 5'ACTCCAAGCAACTCCCACAC3'). Direct sequencing of the PCR product identified two polymorphisms: 1) Nucleotides 1220-1224 (referring to Genbank Acc. No. U10406), C57BL/6J: CCCC; M. spretus: CCCC, 2) Nucleotides 1336 1349: C57BL/6J: GGTGTGTGA-GAGAA and M. spretus: GGTGTGTGAAAGAGAA. Genomic DNA from the panel of 94 backcross animals was PCR-amplified using the aforementioned primers and then hybridized via dotblot analysis with four oligonucleotides: 5'AAGGAAGGGGGACAGG3', 5'AAGGAAGGGGGACAGG3', 5'CTCTCACACA3', 5'CCACACACTTCTCTT3', which specifically bound to each of the four polymorphic sequences, respectively. The resulting allele patterns were compared to those of other loci previously mapped in this cross to detect linkage.

The CPβ gene (*Cappb1*) mapped to Chromosome 4 between *D4Mit16* and *D4Mit13*. Additional linkage information is available at <http://www.jax.org./resources/documents/cmdata>. Mouse Genome Database accession numbers for the mapping data are J:55295 (*Cappb1*).

The order of loci (with recombination distances in centimorgans, cM and standard error) and intergenic distances for the CPβ gene (Figure 1 and 2) is as follows: *Cappb1*: Nearby genes include *Cdc42* and *Afar*. *D4Mit339*- (1.06 ± 1.06 cM) -*D4Bwg0593e*- (1.06 ± 1.06 cM) -*Cdc42*- (1.06 ± 1.06 cM) -*Afar*, *Cappb1*- (4.25 ± 2.08 cM)-*D4Mit312*.

To determine if the *Cappb1* gene was equivalent to a locus that had previously been mapped thereby identifying a possible association with an existing mutant, the Jackson Laboratory Mouse Genome Database (MGD) and the Mouse Chromosome Committee Report (1999) were scanned for loci that lie within 10 cM of the map positions of *Cappb1*. snubnose (*sn*), curly tail (*ct*), and cribriform degeneration (*cri*) are candidate mutants for *Cappb1*.

Jackson BSS Chromosome 4

**Figure 2**

Map from the Jackson BSS backcross showing part of Chromosome 4. The centromere is toward the top. Loci mapping to the same position are listed in alphabetical order. Missing typings were inferred from surrounding data where assignment was unambiguous. Raw data from Jackson Laboratory were obtained from [www.jax.org./resources/documents/cmdata].

The likelihood that *Cappb1* and *sno*, *ct* and *cri* map to the same position was evaluated by determining the confidence intervals associated with their map positions (Figure 3). *Cappb1* (66.8 cM, MGD) was mapped relative to the well-established anchoring locus *D4Mit13* (71 cM, MGD), with 5 recombinants in 94 backcross samples. At 95% confidence, the upper and lower limits are 2.5 and 12.5, respectively [10]. *sno* (58.3 cM, MGD) was mapped relative to tyrosinase related protein, *Tyrp1*, (39 cM, MGD) with 92 recombinants in 535 backcross animals providing confidence limits of 14 and 20.5 [11]. *cri* (69 cM, MGD) was mapped relative to *Tyrp1*, but raw mapping data were not reported, which precludes determination of the confidence limits [12]. *ct* (69 cM) was mapped relative to *D4Mit13* with 26 recombinants in 272 backcross animals providing confidence limits of 6.6 and 13.7. The confidence intervals of *ct* and *Cappb1* overlap, indicating that equivalency of these loci are not ruled out by the mapping data. Because the confidence interval of the mapping of *cri* could not be determined, the equivalency of *cri* and *Cappb1* cannot be ruled out. The confidence intervals of *sno* and *Cappb1* do not overlap, indicating that these loci are not equivalent.

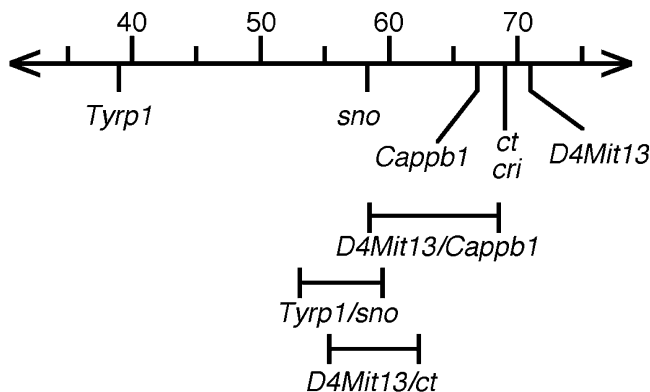


Figure 3
The confidence limits of the mapping of *Cappb1* and its candidate genes: snubnose (*sno*), curly tail (*ct*), and cribriform degeneration (*cri*). The chromosomal region where *Cappb1* and the candidate genes reside is depicted. The MGD map positions are indicated above. 95% confidence intervals based on specific mapping experiments, discussed in the text, are indicated below.

Analysis of the mutant phenotype for *cri* and *ct* is a way to evaluate their potential roles. Mutations in *cri* have a severe neurological defect that can be traced to vacuolar degeneration in white and gray matter of the spinal cord and brainstem [13]. Mutations in *ct* have some degree of spina bifida which results in a curly tail and occasionally, exencephaly [14]. Mutations in *sno* are recognized by their short noses and have spina bifida occulta in their lumbar, posterior thoracic and sacral vertebrae [15]. Ad-

ditional studies will be necessary to determine whether these loci encode or map to *Cappb1*.

Comparative gene mapping between mouse and human has revealed numerous regions of homologous genome organization [16]. The mapping of *Cappb1* supports the evolutionary conservation between the two species. The chromosomal assignment of the mouse CP β gene to Chromosome 4 is consistent with the localization of the human CP β gene to chromosome 1 position p36.1. These regions of the mouse and human chromosomes have conserved synteny.

Conclusions

The CP β gene (*Cappb1*) mapped to Chromosome 4 between *Cdc42* and *D4Mit312*. Three mouse mutations, snubnose, curly tail, and cribriform degeneration, map in the vicinity of the β gene.

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