mations [13]. *wl* is a recessive mutant that is characterized by lethal infantile ataxia, axonal degeneration with secondary myelin dissolution, and low white blood cell counts, resulting in death by the age of four weeks [14,15]. The possibility that *Gata4* is responsible for these phenotypic mutants will need further investigation.

### *References*


1. Rowe, L.B., Nadeau, J.H., Turner, R., Frankel, W.N., Letts, V.A., Eppig, J.T., Ko, M.S., Thurston, S.J., Birkenmeier, E.H. (1994). Mamm. Genome 5, 253–274.
2. Grepin, C., Dagnino, L., Robitaille, L., Haberstroph, L., Antkaly, T., Nemer, M. (1994). Mol. Cell. Biol. 14, 3115–3129.
3. Laverriere, A.C., MacNeil, C., Mueller, C., Poelmann, R.E., Burch, J.B.E., Evans, T. (1994). J. Biol. Chem. 269, 23177–23184.
4. Pevny, L., Simon, S.C., Robertson, E., Klein, W.H., Tsai, S., D'Agati, V., Orkin, S.H., Costantini, F. (1991). Nature 349, 257–260.
5. Dorfman, D.M., Wilson, D.B., Bruns, G.A.P., Orkin, S.H. (1992). J. Biol. Chem. 267, 1279–1285.
6. Tsai, F.Y., Keller, G., Kuo, F.C., Weiss, M., Chen, H., Rosenblatt, M., Alt, F.W., Orkin, S.H. (1994). Nature 371, 221–226.
7. Ho, I.C., Vorhees, P., Marin, N., Oakley, B.K., Tsai, S.F., Orkin, S.H., Leiden, J. (1991). EMBO J. 10, 1187–1192.
8. Ko, L.J., Yamamoto, M., Leonard, M.W., George, K.M., Ting, P., Engel, J.D. (1991). Mol. Cell. Biol. 11, 2778–2784.
9. Arceci, R.J., King, A.A., Simon, M.C., Orkin, S.H., Wilson, D.B. (1993). Mol. Cell. Biol. 13, 2235–2246.
10. Ip, H.S., Wilson, D.B., Heikinheimo, M., Tang, Z., Ting, C., Simon, M.C., Leiden, J.M., Parmacek, M.S. (1994). Mol. Cell. Biol. 14, 7517–7526.
11. Molkentin, J.D., Kalvakolano, D.V., Markham, B.E. (1994). Mol. Cell. Biol. 14, 4947–4957.
12. Nadeau, J.H., McCarthy, L., Cox, R. (1993). Mamm. Genome 4 (suppl.) S203–S210.
13. Hummel, K.P. (1959). Pediatrics 23, 212–221.
14. Dickie, M.M., Schneider, J., Harman, P.J. (1952). J. Hered. 43, 282–286.
15. Luse, S.A., Chenard, C., Finke, E.H. (1967). Arch. Neurol. 17, 153–161.


## Localization of the thyrotropin-releasing hormone gene, *Trh*, on mouse Chromosome 6


**M.L. Roller, S.A. Camper**

Department of Human Genetics, The University of Michigan Medical School, Ann Arbor, Michigan 48109-0618, USA


Received: 3 February 1995 / Accepted: 3 February 1995

***Species:*** Mouse
***Locus name:*** Thyrotropin-releasing hormone
***Locus symbol:*** *Trh*
***Map position:*** *Trh* is localized to mouse Chromosome (Chr) 6: centromere–*D6Mit1*–4.3 ± 2.1–*D6Bir4*–3.2 ± 1.8–*D6Bir5*–8.5 ± 2.9–*Tcrb, D6Nds4*–3.2 ± 1.8–*D6Bir7*–2.2 ± 1.5–*D6Bir8*–2.2 ± 1.5–*D6Mit4*–2.1 ± 1.5–*D6Mit8*–7.4 ± 2.7–***Trh***–6.4 ± 2.5–*D6Bir13*–2.1 ± 1.5–*D6Bir17*–16.0 ± 3.8–*D6Bir19, D6Bir20*–5.5 ± 2.4–*D6Bir23*–telomere.
***Method of mapping:*** *Trh* was localized by haplotype analysis of 94 progeny from an interspecific backcross, (C57BL/6J × *M. spretus*)$F_1$ × C57BL/6[1].

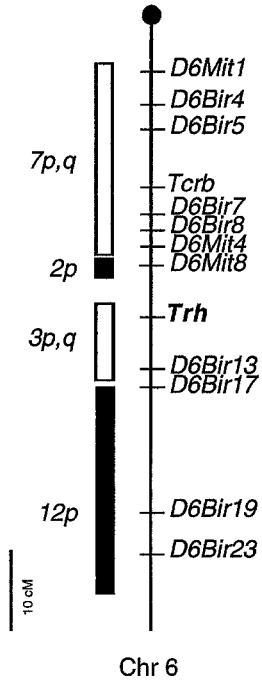



**Fig. 1.** *Trh* maps on mouse Chr 6 in a region with homology to human Chr 3. The position of *Trh* relative to other loci mapped on the same backcross is indicated at the right. The region of synteny homology with human chromosomes were defined with *D6Mit8* as an anchor point for comparison with the consensus map [6]. The positions of *D6Bir13* and *D6Bir17* relative to the HSA3 and HSA12 homologous groups could not be precisely determined.

***Molecular reagents:*** A portion of the *Trh* gene was amplified from C57BL/6J and *M. spretus* genomic DNA by use of the polymerase chain reaction (PCR). Primers designed from the mouse cDNA sequence amplified the last 124 bp of the coding sequence and 471 bp of the 3′ untranslated region (UTR), resulting in a 595 bp product. The sequence of the oligonucleotides was 5′ GTAAGGTTAGAGTCAGGCTTTAGG 3′ forward and 5′ GTTATTTTATATAGGTCCAGTTTTTT 3′ reverse. Restriction digestion of the PCR product with *Nde*I produced the predicted fragments, confirming that the amplification product was *Trh*.
***Allele detection:*** Polymorphisms in the 595 bp PCR product between C57BL/6J and *M. spretus* were detected by single-stranded conformation polymorphism (SSCP). The PCR conditions consisted of 30 cycles of 94°C for 1 min, 61°C for 2 min, and 72°C for 2 min. Polymorphic differences were detected by electrophoresis of the PCR products on a 0.5× Mutation Detection Enhancement (MDE) gel (AT Biochem, Malvern, Pa.).
***Discussion:*** The mouse *Trh* gene encodes a 256-amino acid precursor prepropeptide that is cleaved and processed in the hypothalamus to make the tripeptide TRH (pGlu-His-Pro-$NH_2$, where pGlu is pyroglutamic acid) [2,3]. The precursor contains multiple copies of the TRH progenitor sequence, Gln-His-Pro-Gly, flanked by basic amino acids which are proteolytically cleaved to yield five copies of TRH [2,3]. Even though TRH has been found ubiquitously in the body, the mRNA is expressed only in the hypothalamus and testes. Thyrotropin releasing hormone (TRH) is released from the hypothalamus and regulates the secretion of thyrotropin, (thyroid stimulating hormone, TSH) [4] and prolactin (PRL) [5] from the anterior pituitary gland. TRH activates phosphoinositide metabolism and protein kinase C to cause an increase in intracellular calcium levels resulting in the release of TSH and PRL from the anterior pituitary cells [5]. Mutations in *Trh* would be expected to result in severe hypothyroidism and growth insufficiency beginning at 1–2 weeks of age. The human TRH gene has

*Correspondence to:* S.A. Camper

been mapped to Chr 3 by human–hamster somatic cell hybrid analysis. We localized the mouse *Trh* gene on Chr 6 in a region with homology to the human Chr 3 [6]. There are no known mouse mutants in this region that exhibit hypothroidism or growth insufficiency.

*Acknowledgments:* We thank The Jackson Laboratory for providing the backcross DNA. This work was supported by the National Institutes of Health grant NIH RO1-HD30428 (S.A. Camper).

### *References*

1. Rowe, L.B., Nadeau, J.H., Turner, R., Frankel, W.N., Letts, Y.A., Eppig, J.T., Ko, M.S.H., Thurston, S.J., Benkenmeier, E.H. (1994). Maps from two interspecific backcross DNA panels available as a community genetic mapping resource. Mamm. Genome 5, 253–274.
2. Bulant, M., Roussel, J., Astier, H., Nicolas, P., Vaudry, H. (1990). Processing of thyrotropin-releasing hormone prohormone (pro-TRH) generates a biologically active peptide, prepro-TRH-(160-169), which regulates TRH-induced thyrotropin secretion. Proc. Natl. Acad. Sci. USA 87, 4439–4443.
3. Satoh, T., Yamada, M., Monden, T.M.I., Mori, M. (1992). Cloning of the mouse hypothalamic preprothyrotropin-releasing hormone (TRH) cDNA and tissue distribution of its mRNA. Mol. Brain Res. 14, 131–135.
4. Wilber, J.F., Yamada, M., Kim, U.J., Feng, P., Carnell, N.E. (1992). The human preprothyrotropin-releasing hormone (TRH) gene; cloning, characterization, hormonal regulation, and gene localization. Trans. Am. Clin. Climatol. Assoc. 103, 111–119.
5. Yan, G., Pan, W.T., Bancroft, C. (1991). Thyrotropin-releasing hormone action on the prolactin promoter is mediated by the POU protein Pit-1. Mol. Endocrinol. 5, 535–541.
6. Moore, K.J., Elliot, R.W. (1993). Mouse Chromosome 6. Mamm. Genome 4(Suppl.), S88–S109.

## Assignment of the gene encoding centrosome-associated protein CCD41 to mouse Chromosome 2H

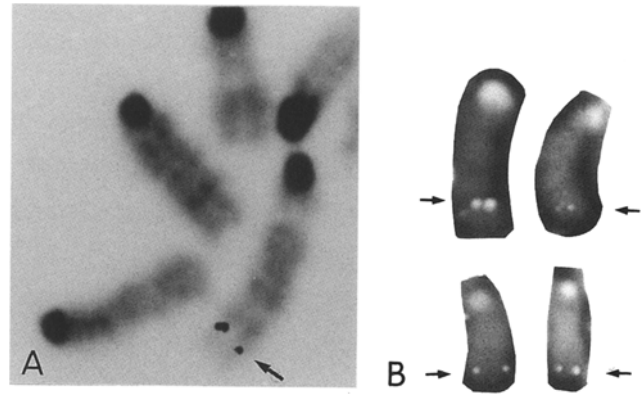
**A. Mincheva,[1] K. Rothbarth,[2] D. Werner,[2] P. Lichter[1]**

[1]Abt. Organisation komplexer Genome, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany
[2]Abt. Biochemie der Zelle, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

Received: 7 November 1994 / Accepted 30 January 1995

***Species:*** Mouse
***Locus name:*** Cell cycle, centrosome associated
***Locus symbol:*** Ccca
***Map position:*** 2H1-3
***Method of mapping:*** Fluorescence in situ hybridization
***Database deposit information:*** EMBL accession number X63748
***Molecular reagents:*** Plasmid pCCD41 containing a 1.349-kb cDNA insert [1] was labeled by nick-translation in the presence of biotin-16-d-UTP, and chromosomal in situ suppression hybridization was performed as described [2] with 60-ng probe DNA and 7 μg of the Cot1 fraction of genomic mouse DNA as competitor in a 10 μl volume. Mouse metaphase spreads were prepared from the spleen cells of a female Balb/c animal according to the published procedure [3]. Hybridized probe was detected by FITC-conjugated avidin, and chromosomes were banded by staining with the DAPI dye. FITC and DAPI images were acquired separately by using a cooled CCD camera, carefully aligned and electronically overlaid.

*Correspondence to:* A. Mincheva




**A.** Section of a mouse metaphase spread after in situ hybridization with the biotinylated probe pCCD41, detected with FITC conjugated to avidin. Arrow indicates the fluorescent signal on Chr 2 within the H1-3 region. Chromosomes were counterstained with DAPI; in panel **A** the DAPI image is inverted to depict the chromosomal bands in a G-banding-like manner, facilitating the assignment of the signal to chromosomal bands. **B.** Examples of hybridization signals on four DAPI-stained Chr 2 homologs.

***Discussion:*** Specific hybridization signals were found on Chromosome (Chr) 2 in the proximal part of bands H1 to H3. Of 39 metaphase spreads examined in detail, two showed signals on both Chr 2 homologs, and 32 exhibited hybridization signals on one of the two Chrs 2. In two metaphases the signal was detected in both Chrs 2; in five spreads a few additional fluorescent spots were seen; however, since they did not occur consistently at a particular chromosomal region, they were considered background. Accordingly, the gene for CCD41 is localized in the proximal part of mouse Chr 2H1-3. CCD41 is a centrosome-associated antigen occupying a compact structure inside the centrosome. It is prevalent in the $G_2$ and M phases of the cell cycle, where it is found not only in centrosomes but also accumulated in perinuclear vesicles [1]. The epitope in the centrosome is exposed throughout the cell cycle except for a brief period immediately after the formation of the daughter centrosomes. On the basis of these observations, it was suggested that the protein plays an essential role in the centrosome function regarding cell cycle progression and control of the three-dimensional structure of the cytoplasm. In accordance with this assumed central function of CCD41, mutations in this gene may result in severe phenotypic abnormalities. Several of such phenotypes are described as genetic traits located within the mouse Chr 2H1-3 including lethal mutations [4,5]. Since the CCD41 gene might provide a candidate gene for some of the corresponding traits, it will be interesting to analyze the expression pattern of CCD41 and to compare it with the various phenotypes of the genetic traits assigned to the mouse Chr 2H1-3 or to human Chr 20q, which is syntenic to this chromosomal region of the mouse genome.

*Acknowledgments:* We gratefully acknowledge the technical help of Sandra Weitz and the support of Prof. Harald zur Hausen.

### *References*

1. Rothbarth, K., Petzelt, C., Lu, X., Todorov, I., Howeig, G., Pepperkok, R., Ansorge, W., Werner, D. (1993). J. Cell Sci. 104, 19–30.
2. Lichter, P., Tang, C.C., Call, K., Hermanson, G., Evans, G.A., Housman, D., Ward, D.C. (1990). Science 247, 64–69.
3. Sawyer, J., Moore, M., Hozier, J. (1987). Chromosoma 95, 350–358.
4. Lyon, M.F., Kirby, M.C. (1994). Mouse chromosome atlas. Mouse Genome 92, 19–107.
5. Peters, J., Cocking, Y. (1994). Mouse gene list. Mouse Genome 92, 169–347.