Short Communications

Mammalian Genome

Mammalian Genome 7, 459–460 (1996).

Linkage mapping of murine homolog of the yeast *SPT6* gene to MMU11B1

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Received: 30 January 1996 / Accepted: 5 March 1996

We have cloned the murine and human homologs of a gene, *SPT6*, that mediates chromatin structure and transcriptional activity in yeast (Chiang et al. 1996). The mammalian *SPT6* homologs are virtually identical as they share >98% identity and >99% similarity at the protein level. The derived amino acid sequences of these two genes both predict a 1726 aa protein with several known features, including a highly acidic 5′-region, and MAPK consensus sites. We mapped the human SUPT6H gene to 17q11.2 and show here that the murine *Supt6h* gene maps to the corresponding region of 11B1.

SPT6 was originally identified in the yeast, *Saccharomyces cerevisiae*. Sixteen mutations that modulate transcription in *S. cerevisiae* were selected as suppressors of *Ty* insertions at HIS4 and LYS2, and were termed the SPT (suppressors of *Ty*) family of genes. Three members of this family in yeast are known: TFIID, histone H2A, and histone H2B. There are two genetically distinct groups of STP genes (TFIID group and histone group), with *SPT4, SPT5*, and *SPT6* belonging to the histone group (Winston 1992). These genes suppress the loss of transcriptional activation of the *SUC2* gene caused by *snf2*, *snf5*, and *snf6* mutations. The SPT5 and SPT6 proteins have highly negatively charged 5′ regions, consistent with the hypothesis that they function in the nucleus, binding to positively charged nucleoproteins to form complexes that mediate chromatin structure. The importance of this subfamily is emphasized by the observation that most double mutant combinations among the *SPT4*, *SPT5*, and *SPT6* genes are lethal in haploid strains (Winston 1992).

The *emb-5* gene in *C. elegans* (Nishiwaki et al. 1993) is a homolog of the yeast *SPT6* gene. *emb-5* is required for gastrulation and for the correct timing of gut precursor cell division. The *hc*61 mutation, a recessive, temperature-sensitive, maternal-effect mutation in the *emb-5* gene, results in the premature entry of E cells at the 26-cell stage into the mitotic (M) phase of the cell cycle. In addition to this early embryonic cell-cycle control, *emb-5* acts during post-embryonic development: growth of *emb-5* mutants (*hc*61) at a nonpermissive temperature from the L1 stage yields adults whose gonadal size is only half of that of the wild type and in whom no mature oocytes are found in the proximal arms of the gonads.

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An intragenic DNA polymorphic marker flanking a dinucleotide repeat in an intron of the murine *Supt6h* gene was isolated by sequencing a PCR product from genomic DNA. The PCR primers were derived from the *Supt6h* cDNA sequence, and the expected size of PCR product from the cDNA sequence is 270 bp. The sequence of the forward primer is 5′-GACATCAGCATAGATTT-GAAGGG-3′ and that of the reverse primer is 5′-GGTGCCAC-TCTCAACCAATT-3′. The PCR program was as follows: denaturing, 92°C (30 s); annealing, 58°C (30 s); extension, 72°C (30 s) for 29 cycles, followed by 10 min at 72°C. The amplified PCR product from genomic DNA was larger that expected from the cDNA sequence, indicating the insertion of an intron. Upon sequencing the PCR product, an intron with a dinucleotide repeat was found. Primers flanking the dinucleotide repeat were synthesized (forward: 5′-GGATCACAAGGTCAAGCCTGGG-3′; reverse: 5′-GTGAACTCGGGTTGTCAGGGCTG-3′).

Supt6h was mapped to mouse Chromosome (Chr) 11 with an intersubspecific backcross of DF/B-*df*/*df* with *Mus musculus castaneus*. The polymorphic region was amplified with the PCR primers flanking the dinucleotide repeat mentioned above. The amplification products, 175 and 193 bp for CASA/Rk and DF/B-*df*/*df* respectively, were separated on 3% agarose gels and visualized by ethidium bromide staining. The backcross, (DF/B-*df*/*df* × CASA/Rk) × DF/B-*df*/*df*, was previously typed for over 20 loci on Chr 11 (Roller et al. 1995). An interval mapping strategy was used to place *Supt6h* approximately on Chr 11. A subset of 97 progeny mice that were typed previously in the interval *Asgr1* to *Thra* were typed for *Supt6h* and *D11Mit35*. No recombinants were detected between *Supt6h* and *Tnfaip1*. Minimization of recombination unambiguously placed *Supt6h* with the following gene order and genetic distance: centromere–*Asgr1, Rpo2-1*–1.0 ± 1.0 cM–(*Tnfaip1, Supt6h*)–2.1 ± 1.4 cM–*D11Mit35*–3.1 ± 1.8 cM–*Tcf2*. These results put *Supt6h* in a region of mouse Chr 11B1 that exhibits extensive synteny homology with human Chr 17.

This subregional mapping on Chr 11 raised the possibility that variations in *Supt6h* could underlie the *Om* (ovum mutant) phenotype. This phenotype is marked by the lethality of embryos conceived by the mating of DDK females with males of other strains, whereas the mating of DDK males with females of other strains gave normal-sized litters (Baldacci et al. 1992). The involvement of the SPT6-homolog *emb-5* in embryogenesis of *C. elegans* supported the hypothesis that mutations in *Supt6h* were

responsible for the *Om* phenotype. This possibility was investigated formally by following segregation of *Om* and *Supt6h* in the backcross progeny of appropriate mating.

BALB/c and DDK mice were from breeding colonies at the Pasteur Institute. The backcross between BALB/c females and (BALB/c × DDX)F_1 males was described (Baldacci et al. 1992). This backcross was continued to the BC6 generation by mating males, heterozygous at the *Om* locus, to BALB/c females (Baldacci et al. 1995); 405 BC individuals were analyzed. The genotype of recombinant backcross males at the *Om* locus was determined by an *in vitro* assay, as described previously (Baldacci et al. 1992). The EUCIB backcross has been described previously (Breen et al. 1994). The LS series ((SPR × C57Bl/6)F_1 × SPR) contains 427 samples of which 207 are recombinants on Chr 11 between the markers *Csfgm* and *D11Mit10*. DNAs from 201 recombinant samples were analyzed with microsatellites in the region around *Om*. The distances in the backcrosses were calculated as the frequency of recombinants with the 95% confidence interval of the distance given in square brackets.

Eight out of 240 progeny carried recombinations between *Supt6h* and *Om*, demonstrating that *Supt6h* was nonallelic, being 3.3 cM [1.45–6.46 cM] proximal to *Om*. *Supt6h* was not separated from the markers *Nos2*, *D11Mit94*, *96*, and *144* in this cross. These markers are therefore at a maximum of 1.25 cM from *Supt6h* at the 95% confidence level. These data were integrated into the map of Chr 11 (Baldacci et al. 1995). The order of markers and the genetic distances at the 95% confidence interval, calculated from the combined data are (oriented from 11 cen → tel):***Supt6h,*** *D11Mit94, 96, 144*–1.67 cM [0.5–4.2 cM]–*D11Mit118*–0.85 cM [0.2–2.5 cM]–*D11Mit33, 93, Scya2*–0.28 cM [0.0–1.6 cM]–*Scya1, Om*–1.55 [0.6–3.3 cM]–*D11Mit35, 36*–0.26 cM [0.0–1.4 cM]–*D11Mit38, 39, Mpo*.

The segregation of *Spth6* and several Chr 11 markers was also tested on the EUCIB backcross to obtain an independent map and more precise mapping. The calculated distances between the markers were: *Csfgm*–8.4 cM [6.0–11.5 cM]–*D11Mit30*–0.23 cM [0.0–1.3 cM]–*Atp1b2, D11Mit31*–1.4 cM [0.5–3.0 cM]–***Supt6h,*** *Nos2, D11Mit94, 96, 144*–0.23 cM [0.0–1.3 cM]–*D11Mit92, 95*–0.47 cM [0.0–1.7 cM]–*D11Mit33, 247*–0.47 cM [0.0–1.7 cM]–*D11Mit37, Scya1, Scya2*–0.47 cM [0.0–1.7 cM[–*D11Mit36*–36.5 cM [32–41.3 cM]–*D11Mit10*. The absence of recombinants in the 427 samples analyzed indicated that *Supt6h* is at a maximum of 0.7 cM (95% confidence level) from the markers *Nos2, D11Mit94, 96*, and *144*. In summary, fine mapping shows that *Supt6h* is not the *Om* locus, but it maps *Supt6h* more precisely on mouse Chr 11.

These genetic data indicated that *Supt6h* mapped into the region bounded by *D11Mit31–D11Mit92* and that it was tightly linked to *Nos2, D11Mit94, 96*, and *144*. *Nos2, D11Mit94, 96*, and *144* are all located on a YAC contig that spans most of the region *D11Nds1* to *D11Mit8* (C.C. Blackburn, G. Morahan, and A. Ashworth, unpublished; Nehls et al. 1994). YACs were identified by PCR screening of YAC DNA pools from the St. Mary's Hospital mouse YAC library and the Princeton University YAC library resource, or were obtained from Research Genetics (Huntsville, Ala.). The resulting YAC contig (C.C. Blackburn, G. Morahan, and A. Ashworth, unpublished) was probed by PCR with the *Supt6h* specific primers described above. PCR amplifications were performed with γ-[^{32}P] ATP (Amersham) end-labeled primers as described (Blackburn et al. 1994). Reactions were run in a final volume of 10 μl under the following PCR conditions: denaturing, 94°C (60 s); annealing, 55°C (60 s); extension, 72°C (30 s); 35 cycles, before analysis on 4% denaturing TBE-polyacrylamide gels, followed by autoradiography. This analysis showed that *Supt6h* was present on YAC 61F12 but not on YAC 159D9, placing it between markers *D11Bhm153* and *D11Bhm154* (Nehls et al. 1995).

The mouse *Supt6h* gene maps into a region of considerable interest on MMU11, tightly linked to the mutations nude (*nu;* Takahasi et al. 1992), open eyelids (*oe;* Kelton and Rauch 1968), and ovum mutant (*Om;* Baldacci et al. 1992). The pleiotropic function described for homologs of this gene in lower eukaryotes suggests that *Supt6h* would be a candidate for any of these mutations. Our data clearly exclude *Supt6h* as a candidate for *Om*, as *Supt6h* was segregated from this phenotype in an *Om* backcross. Other investigators have screened this region of MMU11 for novel genes using both direct cDNA selection and exon trapping techniques. Using direct cDNA selection, Segre and associates (1995) detected a cDNA fragment with homology to *emb-5* on a mouse YAC that also contained the nude locus. Their data placed what we now know to be *Supt6h* approximately 100 kb from *whn*, which was recently identified as the candidate for the nude mutation (Nehls et al. 1994). This serendipitous finding is consistent with our extensive genetic and physical mapping, which mapped the *Supt6h* gene to YAC 61F12, within the minimum genetically defined nude locus (Segre et al. 1995). 61F12 also carries the *whn* gene (Nehls et al. 1994). The potential involvement of *Supt6h* in the open eyelids phenotype is being investigated.

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