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YAC Contigs of the *Rab1* and *wobbler* (*wr*) Spinal Muscular Atrophy Gene Region on Proximal Mouse Chromosome 11 and of the Homologous Region on Human Chromosome 2p

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Despite rapid progress in the physical characterization of murine and human genomes, little molecular information is available on certain regions, e.g., proximal mouse chromosome 11 (Chr 11) and human chromosome 2p (Chr 2p). We have localized the *wobbler* spinal atrophy gene *wr* to proximal mouse Chr 11, tightly linked to *Rab1,* a gene coding for a small GTP-binding protein, and *Glns-ps1,* an intronless pseudogene of the glutamine synthetase gene. We have now used these markers to construct a 1.3-Mb yeast artificial chromosome (YAC) contig of the *Rab1* region on mouse Chr 11. Four YAC clones isolated from two independent YAC libraries were characterized by rare-cutting analysis, fluorescence *in situ* hybridization (FISH), and sequence-tagged site (STS) isolation and mapping. *Rab1* and *Glns-ps1* were found to be only 200 kb apart. A potential CpG island near a methylated *Nar*I site and a trapped exon, *ETG1.1,* were found between these loci, and a new STS, *AHY1.1,* was found over 250 kb from *Rab1.* Two overlapping YACs were identified that contained a 150-kb region of human Chr 2p, comprising the *RAB1* locus, *AHY1.1,* and the human homologue of *ETG1.1,* indicating a high degree of conservation of this region in the two species. We mapped *AHY1.1* and thus human *RAB1* on Chr 2p13.4–p14 using somatic cell hybrids and a radiation hybrid panel, thus extending a known region of conserved synteny between mouse Chr 11 and human Chr 2p. Recently, the gene *LMGMD2B* for a human recessive neuromuscular disease, limb girdle muscular dystrophy type 2B, has been mapped to 2p13–p16. The conservation between the mouse *Rab1* and human *RAB1* regions will be helpful in identifying candidate genes for the *wobbler* spinal muscular atrophy and in clarifying a possible relationship between *wr* and *LMGMD2B.*

INTRODUCTION

The construction of yeast artificial chromosome (YAC)-based physical maps of genomic regions, in conjunction with chromosomal mapping based on segregation, is a powerful tool to advance the identification of gene complexes and of disease genes. In this respect, the analysis of human chromosomes 16 and 19 (Nowak, 1995) and mouse chromosomes 1 (Hunter *et al.,* 1994) and 17 (Cox *et al.,* 1993) as well as of human and murine X chromosomes is particularly far advanced (Hamvas *et al.,* 1993). On the other hand, such extensive information is not available for mouse proximal chromosome 11 (Chr 11) and human chromosome 2p (Chr 2p) (Fig. 1; cf. Berry *et al.,* 1995; Nowak, 1995), known to share at least the genes for the reticuloendotheliosis oncogene (Brownell *et al.,* 1985), for a brain-specific β-spectrin isoform (Bloom *et al.,* 1992), and for cytoplasmic malate dehydrogenase (Ball *et al.,* 1994). However, comparing the segregation map of the mouse with the human cytogenetic map, a colinear relationship cannot be established: The positions of the reticuloendotheliosis oncogene and spectrin gene appear to be interchanged between the two species (Fig. 1).

In the mouse, this region was further characterized by being shown to contain *Rab1,* a gene coding for a small GTP-binding protein, and *Glns-ps1,* a glutamine synthetase pseudogene (Wichmann *et al.,* 1992). Our interest in this chromosomal region relates to the investigation of a mouse spinal muscular atrophy mutation, *wobbler* (*wr;* Duchen and Strich, 1968), which we have localized to proximal Chr 11 (Kaupmann *et al.,* 1992). This localization has led to the consideration and exclusion of several candidate genes and the identification of human Chr 2p as the region in which the homologue of *wr* should be located (Kaupmann *et al.,* 1991; Lengeling *et al.,* 1994). At present, there are three genes for which no recombination with the *wr* locus has been found among 54 backcross animals showing the wobbler (WR) phenotype: *Rab1, Glns-ps1* (Wichmann *et al.,* 1992), and *Mor2* (Korthaus *et al.,* 1996).

Here, we report on the construction and characterization of a 1.3-Mb mouse YAC contig comprising, in addition to *Rab1* and *Glns-ps1,* a novel trapped exon *ETG1.1.* Human *RAB1* was mapped to Chr 2p and used to construct a 1.2-Mb YAC contig for detailed homology studies.

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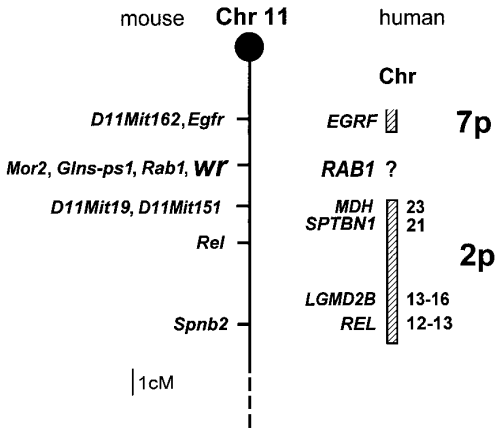

FIG. 1. The genetic map around the *Rab1*/*wr* locus on proximal mouse Chr 11 and homologous regions on human Chr 7 and Chr 2. Gene symbols *Egfr*/*EGFR*, epidermal growth factor receptor; *Mor2*/*MDH*, cytoplasmic malate dehydrogenase; *Glns-ps1*, glutaminesynthetase pseudogene 1; *Rab1*/*RAB1*, *ras*-related protein 1; *Rel*/*REL*, reticuloendotheliosis oncogene; *Spnb2*/*SPTBN1*, brain-specific isoform of β spectrin; *wr*, wobbler; *LGMD2B*, limb girdle muscular dystrophy type 2B. For references see the Introduction.

MATERIALS AND METHODS

YAC library screening and DNA preparation. Murine and human YACs were isolated from three different genomic libraries constructed at MIT (Kusumi *et al.*, 1993) and ICRF (two libraries: Larin *et al.*, 1991; S. Meier-Ewert, unpublished). The ICRF libraries were screened by hybridization with a 0.87-kb cDNA fragment representing the 3′-nontranslated region of the Rab-1 mRNA (Wichmann *et al.*, 1989). The MIT library was screened using PCR assays of a hierarchy of pooled DNA samples (Green and Olson, 1990). The loci and primer sequences used for PCR screening as well as the PCR conditions are listed in Table 1.

YAC clones were grown in AHC medium (deficient in uracil and tryptophan), and their DNAs were isolated according to standard methods (Polaina and Adam, 1991). Total yeast DNA was prepared in agarose plugs using a lithium dodecyl sulfate method (Anand and Southern, 1990).

Restriction digest YAC mapping. Restriction maps of the YACs were generated by digestion with rare-cutting restriction endonucleases. YACs were digested by incubating the agarose plugs with 30 units of enzyme (Gibco BRL) for 2 h under conditions recommended by the manufacturer. Fragments were sized using a rotating field electrophoresis system (Rotaphor, Biometra) on 1% agarose (Gibco BRL), 0.25× TBE pulsed-field gels using the following switching times: 5 to 90 s (lin ramp), 120 to 180°C (log ramp), 120 to 180 V (log ramp), 13°C for 37 h.

Southern blots were hybridized with pBR322 fragments corresponding to the pYAC4 right (URA arm probed with the 1.7-kb *Pvu*II- and *Bam*HI-digested fragment) and left (TRP arm probed with the 2.7-kb *Pvu*II- and *Bam*HI-digested fragment) vector arms. The Glns and Rab1 probes were prepared as described (Bhandari *et al.*, 1988; Wichmann *et al.*, 1989).

IRS probe generation. Interspersed repetitive sequence polymerase chain reaction (IRS-PCR) was carried out on YAC miniprep DNA, using a B1 (left) primer (ACTCAGAAATCYRCCTGCCTCTGCCTC; Schalkwyk, London, pers. comm., 1994) for murine and an *Alu*-3′-primer (CACCTGAGGTCAGGAGTTC; Bates *et al.*, 1992) for human clones. The PCR cycler (TRIO Thermocycler, Biometra) conditions were 1 cycle at 94°C for 4 min and 35 cycles at 68°C (B1) or 52°C (*Alu*) for 1 min, 72°C for 3 min, and 90°C for 1 min. 3′-Overhanging nucleotides were removed by *Pfu* DNA polymerase treatment (PCR Polishing Kit, Stratagene) followed by phosphorylation using T4 kinase (Gibco BRL). Blunt-ended PCR products were cloned into a *Sma*I-digested pUC 18. To create new sequence-tagged sites (STS), cloned IRS-PCR products were sequenced with pUC 18-specific primers using a T7 DNA sequencing kit (Pharmacia).

Isolation of YAC end fragments. End rescues were carried out by an inverted PCR described as genomic end rescue PCR (Silverman *et al.*, 1989). One microgram of YAC miniprep DNA was digested with either *Eco*RV or *Hae*III, followed by circularization under standard ligation conditions. Subsequently, PCR with pYAC4-specific inverted primers was performed with 50 pmol of each primer, 150 ng circularized DNA, 3 units *Taq* DNA polymerase (Promega), nucleotides, and buffer (as recommended by the supplier) in a total volume of 50 μl. After the first denaturation (94°C for 4 min), 35 cycles of annealing (62°C for 1 min), extension (72°C for 2 min 30 s), and denaturation (90°C for 1 min) were performed. PCR products were cloned into a *Sma*I-digested pUC18 and sequenced as described above.

Exon trapping. YAC ymWIBR141E1 was isolated from the host yeast by PFGE in LMP–agarose (Gibco BRL) and simultaneously digested with 90 units of *Bgl*II and *Bam*HI. After purification with GeneClean (BIO 101), the DNA was cloned into the pSPL1 vector at the *Bam*HI site. Plasmid DNA was prepared by alkaline lysis purified on Qiagen columns and used to transfect COS-7 cells. Cytoplasmic RNA isolation, first-strand cDNA synthesis, and PCR amplification were performed according to Buckler *et al.* (1991). The resulting PCR products were cloned in pUC18 and sequenced as described above for STS generation.

Fluorescence in situ hybridization (FISH). Chromosome spreads were prepared from the WMP mouse cell line, in which all autosomes except Chr 19 are present as metacentric Robertsonian translocations. B1-PCR products of the YACs were labelled with biotin-11–UTP by nick-translation (Bio-Nick Labeling System, Gibco BRL). The probes were preannealed with mouse Cot-1 DNA (Sigma) to suppress the hybridization of repetitive sequences. Hybridization with 100 ng labelled DNA in 20 ml mix per slide was performed at 37°C for 15 h. After *in situ* hybridization and washing procedures, signals were detected with avidin–FITC and anti-avidin-antibody (Vector). Chromosomes were stained with DAPI (1 mg/ml) and propidium iodide (0.5 mg/ml). These experiments were performed in collaboration with Dr. Hameister and C. Klett (Ulm).

Cell hybrid mapping. For the chromosomal localization of the human *RAB1* gene, a Rab1 mouse cDNA subfragment was hybridized to *Bam*HI-digested DNA from 20 human/rodent somatic cell hybrids (Bios Laboratories, New Haven, CT). Subchromosomal localization was determined using total chromosome 2 (NA 108268) and the deletion derivatives GM 11172 and NA 11444 (Coriell Cell Repositories, Camden, NJ), representing the long and the short arm of Chr 2, respectively.

For a more detailed mapping, the GeneBridge 4 radiation hybrid mapping panel (Research Genetics, Huntsville, AL) was used. This panel represents 91 (radiation hybrid) clones of the whole human genome (Walter *et al.*, 1994).

RESULTS

Isolation and characterization of mouse YACs. Two markers, *Rab1* and *Glns-ps1*, known to be closely linked to the *wobbler* (*wr*) locus on proximal mouse Chr 11 (Kaupmann *et al.*, 1992; Wichmann *et al.*, 1992), were used to identify YAC clones derived from that region by screening a total of 40,000 clones in three independent libraries. Using PCR screening to detect a 5′ microsatellite of the *Glns-ps1* pseudogene, a 650-kb YAC, ymWIBR141E1, was identified in the MIT/Whitehead mouse YAC library (Kusumi *et al.*, 1993). A PCR-based screening for a STS from the 3′-UTR of *Rab1* detected a 320-kb YAC, ymWIBR168H4, that was shown to also contain *Glns-ps1*, thus confirming the close genetic (Wichmann *et al.*, 1992) and physical (Meyer-Kleine and Jockusch, unpublished) linkage of

TABLE 1

PCR Primers for STS from the *Rab1/RAB1* Regions

STS	Sequence (5′–3′)	Size (bp)	Annealing	Reference
D11Mit79	TTCTTGGTCGTAGCCCTCAC GACACACAACACCTCGCG	152	62°C	MIT
Glns-ps1	AGCTTTGGAGACAACAATTAGATC TGTTCATCAGCTGAGGAATGGATG	181	60°C	Bhandari *et al.* (1989)
Rab1	GTACTACCTGCTAAACCGTAGGC CTTTCCTGGCCTGCTGTGTCC	246	60°C	Wichmann *et al.* (1990)
BY1.1	CCATACCTTCTGCTGGAGGTATAC CTAGTAGTTGCTCAGTCCCATGAG	185	70°C	This work
BY1.2	CTTCTCTGGTTTTGTAGG CCATTGAGCCTCCTCTATC	62	52°C	This work
ETG1.1	GACAGCTGCTCCATGCGTCC GAGTCAGTTGTCTGAAAAATGCAG	114	56°C	This work
Y1L	CTGTTAGTAGGAAGCAAGGTCTC GATCGCGTAGTCGATAGTGGC	104	66°C	This work
BY2.1	CCTGACCCACTTTCCTACATC GTAGCTGACCTTCTAGTCTTTG	450	64°C	This work
BY2.3	CTCTAGCAGAGGACCCAGGTTC CTGAAAGTGCCGACTACTGTCG	230	68°C	This work
AHY1.1	CAGGCTAAGCTGCCTCTGAC GACAGCTTTTCAGAGCTACTCC	270	65°C	This work

Note. Size, size of the amplification product using C57BL6/J DNA.

Rab1 and *Glns-ps1*. By hybridization screening with a cDNA Rab1 probe, two positive YACs were detected among 5000 clones of the ICRF mouse YAC library (S. Meier-Ewert, unpublished): 903A0745 (550 kb) and 903H082 (1100 kb). Both lacked *Glns-ps1* sequences.

These four mouse YAC clones were tested for chimerism by FISH to mouse metaphase chromosomes and by restriction analysis. The 650-kb YAC, ymWIBR141E1, was chimeric according to FISH analysis, as hybridization signals were seen on Chr 11 and proximal Chr X. The other three YACs appeared non-chimeric, as they gave a signal only on proximal Chr 11 (Table 3).

Restriction analysis was performed using six rare-cutting restriction nucleases and probes for the "left end" (pBR322, 2.7 kb) and "right end" (pBR322, 1.7 kb) inserts, for the STSs *ETG1.1* and *AHY1.1*, and for *Glns-ps1* and *Rab1* (Fig. 2, Table 2). Extensive overlap of the restriction patterns allowed for the construction of a ⩾1.3-Mb contig (Fig. 3), considering that the left arm of YAC ymWIBR141E1 is not derived from Chr 11. The distance between *Glns-ps1* and *Rab1*, which are both

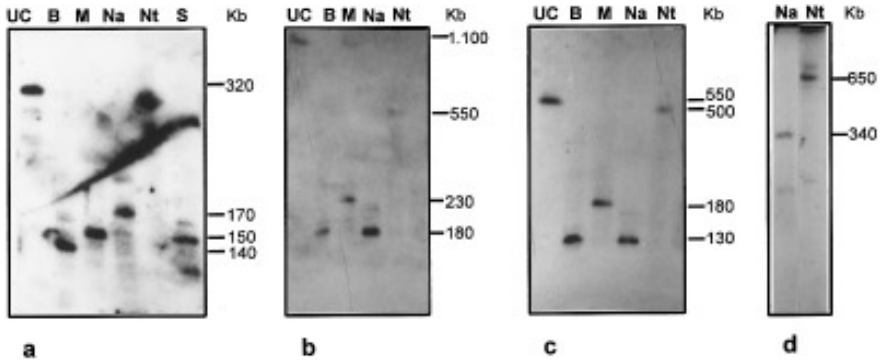

FIG. 2. Rare-cutter restriction patterns of the mouse *Rab1* region on Chr 11. Pulsed-field gel electrophoresis (PFGE) of three different YAC clones ((a) ymWIBR168H4; (b) 903H082; (c) 9030745) and genomic DNA ((d) C57BL/6 kidney). UC, uncut; B, *Bss*HII; M, *Mlu*I; Na, *Nar*I; Nt, *Not*I; S, *Sfi*I. Hybridization patterns with the Rab1 probe are shown as examples. The grouping of fragment sizes in a around 160 kb indicates CpG islands. Comparison of b and c shows common fragments. Comparison of b and c with d shows smaller *Not*I fragments in YAC than in genomic DNA, indicating a *Not*I site near *Glns-ps1* in the genomic DNA. Comparison of a, b, and c with d shows smaller *Nar*I fragments in YACs than in genomic DNA, indicating methylation of a cleavage site in the latter.

TABLE 2

YACs from the *Rab1/RAB1* Regions and Their Rare-Cutter Restriction Fragments

Species	YAC	Size	Probe	*Bss*HII	*Mlu*I	*Nar*I	*Not*I	*Nru*I	*Sfi*I
Mouse (MIT)	ymWIBR141E1	650	Left arm	280	280	140	650		150
			Right arm	270	150	/	650		60
			Glns	270	150	170	650		140
(MIT)	ymWIBR168H4	320	Left arm		150	150	320	150	90
			Right arm		130	5	320	120	20
			Glns	130	150	/	320	110	110
			Rab1	140	150	170	320	150	150
(ICRF)	903H082	1100	Left arm	130	130	130	600	160	130
			Right arm	95	110	80	90	150	90
			Rab1	150	200	150	600		/
			AHY1/1	50	60	110			110
(ICRF)	903A0745	550	Left arm	130	130	130	550	160	/
			Right arm	100	120	90	50		/
			Rab1	130	180	130	500		/
Human (ICRF)	900A0894	1000	Left arm		90			280	
			Right arm		110			210	
			Rab1		150		230	170	
			AHY1.1		150		70		
(ICRF)	900F0982	600	Right arm		90			150	
			Left arm		380			350	
			Rab1		180		230	150	
			AHY1.1		180		210		

Note. Blank space, not done; /, not detected.

contained in YAC ymWIBR168H4, was determined to be 200 kb (Fig. 3).

Correlation to the genomic physical map. Whereas the small physical distance between *Rab1* and *Glns-ps1* confirmed previous genetic and physical evidence, there are two inconsistencies between the YAC map and the genomic restriction map. First, *Rab1* was found on a 650-kb *Not*I fragment of a genomic digest (Meyer-Kleine and Jockusch, unpublished). This would have to extend for about 240 kb into the right arm of ymWIBR141E1. However, no *Not*I site was found in this YAC. In accordance with the FISH result, this argues for a chimerism of this YAC, with the fusion site close to the overlap with the right arm of ymWIBR168H4. This would define the length of the Chr 11 YAC contig as about 1.3 Mb, i.e., the minimum length given above (Fig. 3).

Another discrepancy between the restriction pattern

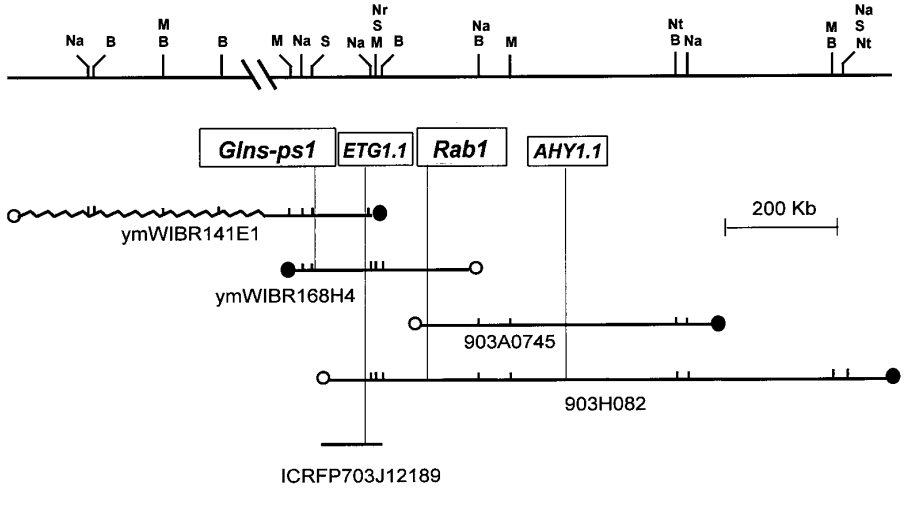

FIG. 3. Mouse YAC contig and rare-cutting restriction map around the *Rab1* gene on Chr 11. Restriction sites: B, *Bss*HII; M, *Mlu*I; Na, *Nar*I; Nt, *Not*I; Nr, *Nru*I; S, *Sfi*I. Positions of restriction sites were determined by hybridization with Glns, Rab1, and probes specific for left (open circle) or right (solid circle) ends of the pYAC4 vector. The extent of overlap between clones was determined by comparison of restriction site patterns and by STS content analysis as shown in Table 3. The positions of *Glns-ps1, ETG1.1, Rab1,* and *AHY1.1* are defined by the nearest restriction sites.

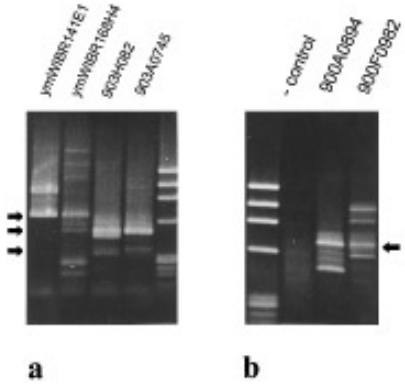

FIG. 4. IRS-PCR of mouse (a) and human (b) YACs from the *Rab1*/*RAB1* regions. Total yeast DNAs containing mouse or human YACs were amplified using B1- and *Alu*-specific primers, respectively. Products were fractionated on a 1.5% agarose gel and stained with ethidium bromide. Overlapping PCR products are indicated by arrows.

of the contig and the genomic one is the presence of a 180-kb *Nar*I *Rab*1-containing fragment in the former and a 340-kb fragment in the latter (Fig. 2). As the relevant stretch of DNA is represented two- to threefold in the YAC, chimerism is excluded as an explanation. Considering the methylation sensitivity of *Nar*I, the most likely explanation is that the *Nar*I site between *Glns-ps1* and *Rab1* is methylated and hence protected in the mouse kidney DNA used. Two putative CpG islands were found in the contig, one near the methylation-sensitive *Nar*I site, and the other near the right end of YAC 903H082 (Fig. 3).

Final establishment of the mouse contig and new markers. Using B1 repetitive element-based PCR, characteristic amplicon band patterns could be established for each YAC (Fig. 4). The YAC ymWIBR168H4 containing the markers *Glns-ps1* and *Rab1* showed the highest abundance of B1 elements (Fig. 4), indicating high gene density in this region (Bickmore and Sumner, 1989).

B1-PCR products were used to create four new physical landmarks. Seven amplification products were cloned in a shotgun manner and were sequenced. Two STSs were detectable on all four YACs and were classified as repetitive. One STS did not show any PCR product using mouse total DNA (C57BL/6J). The remaining four STSs as well as the four STSs *D11Mit79, Glns-ps1, Rab1,* and *EGT1.1* were ordered within the contig (Table 3). The STSs *Glns-ps1, BY1.1,* and *EGT1.1* were ordered using a *BY1.1*-positive 100-kb P1 clone, ICRFP703J12189. The STS *Y1L* constructed by YAC end isolation gave final proof of the orientation of ymWIBR168H4, the restriction pattern of which was ambiguous, due to near symmetry of the cleavage sites. The physical order of the STSs *Rab1* and *BY1.2* could not determined. *D11Mit79* and *BY2.3* are the outermost STS markers of the mouse contig, flanking a region of approximately 600 kb.

Mapping of the human RAB1 gene. By hybridization of the mouse 0.87-kb cDNA RAB1 probe to *Bam*HI digests of a somatic cell hybrid panel, human *RAB1* was mapped to Chr 2. An *Alu*-PCR product that is detectable on both human YACs was used to construct a new STS, *AHY1* (Figs. 4b and 5b). This new marker was localized by restriction analysis and hybridization at a distance of about 70 kb to *RAB1*. In turn, *AHY1.1* was used as a PCR marker on deletion derivatives of Chr 2 and a radiation hybrid panel (GeneBridge4). *AHY1.1* was mapped to Chr 2p13.3–p14, 5.76 cR_{3000} from the STS D2S2090 with a lod score of >3.

The Rab1 probe was used to search for human YACs, and two *RAB1*-containing YACs, 900A0894 and 900F0982, were identified among 10,000 screened clones. Their restriction patterns and relative positions are shown in Fig. 6. The identification of two loci, *ETG1.1* and *AHY1.1*, flanking the *RAB1* locus allowed for the orientation of the human overlapping YACs relative to the mouse YAC contig (Fig. 7).

DISCUSSION

Our YAC study describes, for the genomes of mouse and human, the physical structure of the DNA around the gene for the ubiquitously expressed and highly conserved GTP-binding protein Rab-1. The characterized region comprises, or is in the immediate neighborhood of, the spinal atrophy gene *wobbler* (*wr*) of the mouse and is highly conserved between mouse and human (Fig. 7).

TABLE 3

Characterization by STS Content of Four Mouse YACs, One Mouse P1 Clone, and Two Human YACs from the *Rab1/RAB1* Regions

Species	Clone	Name	FISH	D11Mit79	Glns-ps1	BY1.1	ETG1.1	Rab1	BY1.2	Y1L	AHY1.1	BY2.1	BY2.3
Mouse	YAC	ymWIBR141E1	c	+	+	+	+	0	0	0	0	0	0
		ymWIBR168H4	nc	0	+	+	+	+	+	+	0	0	0
		903H082	nc	0	0	0	+	+	+	+	+	+	+
		903A0745	nc	0	0	0	0	+	+	+	+	+	0
	P1	703J12189	nt		0	+	+	0	0				
Human	YAC	900F0982	nt					+			+		
		900A0745	nt					+			+		

Note. +, present; 0, no signal; c, chimeric; nc, nonchimeric; nt, not tested; blank space, not relevant.

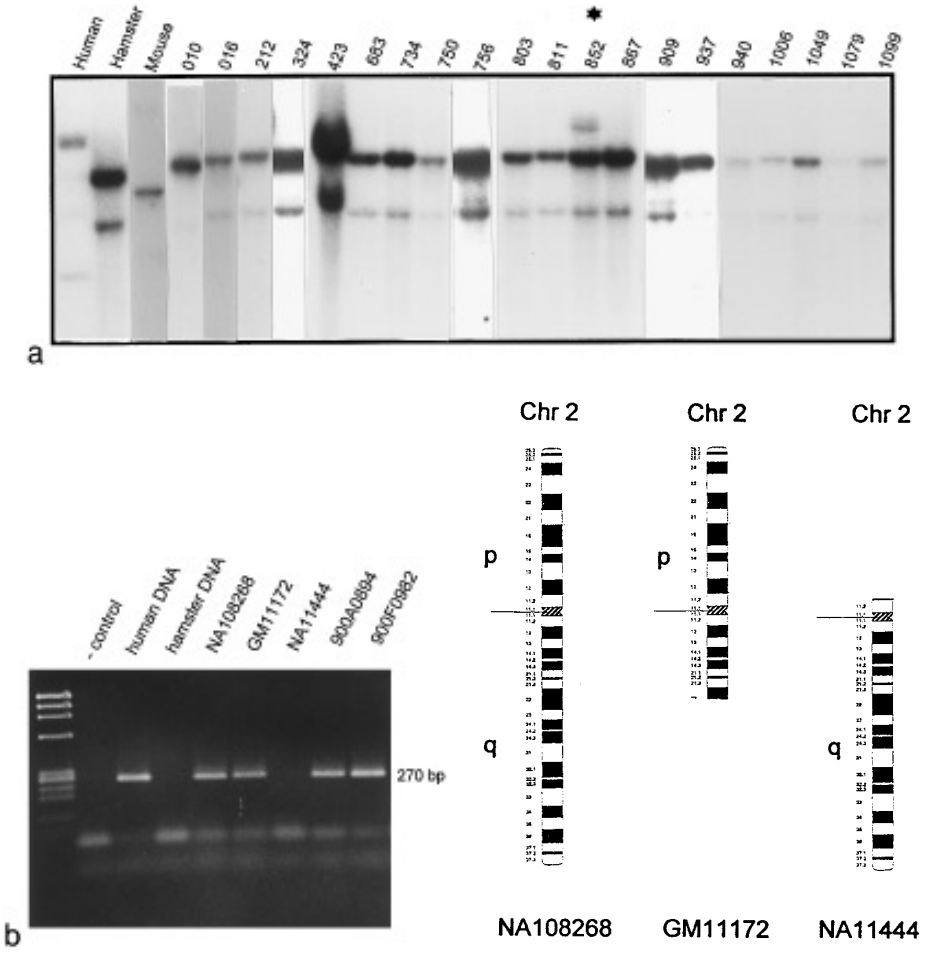

FIG. 5. Mapping of the human *RAB1*. (a) Southern blot using reference DNAs from human, hamster, and mouse as well as DNAs from 20 human/hamster hybrid cell lines. Ten micrograms of DNA digested with *Bam*HI were electrophoresed, blotted, and hybridized with a mouse cDNA Rab1 probe. Hybrid cell line 852 (asterisk) was the only cell line that contained human Chr 2; it contained no other human chromosome. The blot thus shows that *RAB1* maps to human Chr 2. (b) Subchromosomal localization of *RAB1* using PCR on a physically linked STS, *AHY1.1* (cf. Table 3). Lanes from left to right: chain length marker, ϕX174 DNA, *Hae*III digested; control, no DNA; total human DNA; total hamster DNA; NA108268, hamster/human hybrid cell line, containing intact human Chr 2; GM11172, cell hybrid deletion derivate containing human Chr 2p and proximal part of q, as shown to the right; NA11444, deletion derivate containing a proximal fragment of human Chr 2p and Chr q; 900A0894 and 900F0982, two human YACs (cf. Table 3) containing *RAB1*. Two hundred nanograms of DNA was used for each PCR.

What is the relation of the wr gene to the Rab1 contig? The contig around *Rab1* is a starting point for the search for the *wr* locus, as flanking markers narrow the interval for *wr* to 3.1 cM around *Rab1*/*Gln*s-*ps1*. Among 129 F2 individuals with the WR phenotype, we found no recombination with *D11Mit79*, which is located within our *Rab1* contig. Recently, 3 of 235 progeny in a C57BL-*wr*/NZB hybrid panel have been re-

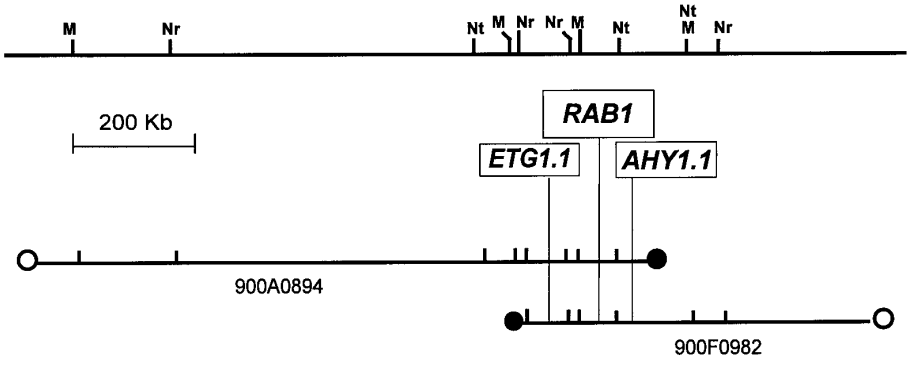

FIG. 6. Human YACs from the *RAB1* region on Chr 2p. Restriction sites given above. Abbreviations as in Fig. 2, except that human gene symbols are in capitals.

ported to show "recombination" between *wr* and *Glns-ps1* (Des Portes *et al.,* 1994). We have found one such case among 19 (C57BL-*wr* × NZB) F2 and F3 individuals. If these observations on phenotypic wildtype mice were due to recombination, the reported numbers would indicate a distance of ⩾1 cM or ⩾2 Mb between *wr* and *Glns-ps1* and therefore between *wr* and *Rab1.* However, these recombinations need to be verified for two reasons. First, only recombinants without the WR phenotype have been reported so far. Second, the markers *D11Mit162* and *D11Mit19* that flank *wr* and *Glns-ps1* in our recombinant are not recombined. A double recombination within a distance of <2 cM would be extremely unlikely. Thus, the recombinants in (C57BL-*wr* × NZB) crosses, defined so far only by the absence of the WR phenotype, may actually represent a suppression of the phenotype by modifier genes derived from the NZB strain. This hypothesis is supported by an observed heterogeneity in neurological and testicular symptoms in the progeny of these crosses (Augustin and Jockusch, in preparation).

Candidate genes for wr. We have tested the possibility that *Rab1* might be identical to the *wr* gene. Using genomic PCR and SSCP analysis, no difference between wildtype and *wobbler* DNA was detected (N. Wedemeyer, unpublished results). Due to a number of stop codons, the standard allele of the pseudogene *Glns-ps1* cannot give rise to a protein (Bhandari *et al.,* 1990). If at all, an abnormal mutant form of the pseudogene would be expected to exert dominant effects. Finally, enzyme electrophoretic patterns of cytoplasmic malate dehydrogenase have been compared between wildtype and wobbler mice. In the eight organs tested (including spinal cord and testis), no difference was found (Korthaus *et al.,* 1996). Thus, for all three genes, *Rab1, Glns-ps1,* and *Mor2,* it is unlikely that they are directly involved in the wobbler disease.

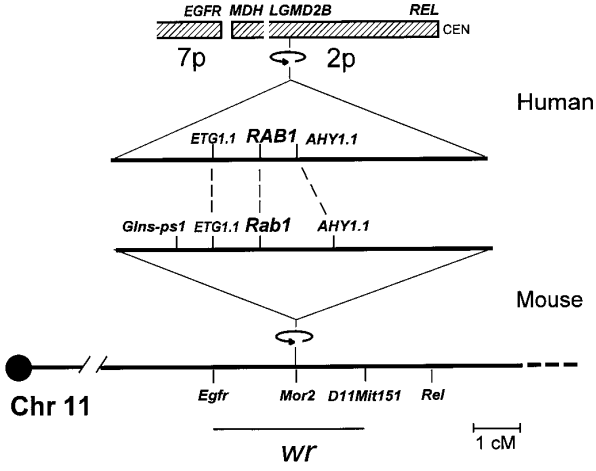

FIG. 7. Synopsis of the *Rab1*/*RAB1* regions on mouse proximal Chr 11 and human Chr 2p. The physical maps of the YAC contigs and their homology are shown in the middle. At the present resolution, these contigs represent loci on the combined genetic/cytogenetic map of human and the genetic map of the mouse and can therefore not be oriented with respect to the centromere (indicated by relating areas). Gene symbols are as in Fig. 1.

Gene content and conservation of the Rab1/RAB1 region. The 350-kb region around *Glns-ps1* and *Rab1* seems to harbor a high density of genes by the following criteria: (1) It contains a putative CpG island; (2) in this region there is at least one methylation site (as defined by *Nar*I cleavage); (3) a wide variety of B1-based PCR products was obtained from this region; (4) at least one exon, *ETG1.1,* could be isolated from this region by exon trapping; (5) the mouse homologue of a human STS, *AHY1.1,* originally identified in human *RAB1*-positive YAC clones, was also found in this region. The order *ETG1.1–Rab1/RAB1–AHY1.1* is found in mouse and human with similar (but not identical) physical distances, indicating a high degree of conservation between these two species (Fig. 7).

We have extended the known region of conservation between mouse Chr 11 and human Chr 2p by the three loci *RAB1* and the newly discovered *ETG1.1* and *AHY1.1.* In the wider genomic context of these, a final clarification of the order of the loci between *REL* and *MDH* and of the possible conservation of gene order in addition to synteny will only be possible on the basis of a more detailed linkage analysis of the human markers.

Possible homology of LGMD2B and wr. Radiation hybrid mapping has placed the human *RAB1* gene at Chr 2p13.3–p14, thus in close proximity to the gene *LGMD2B* for limb girdle muscular dystrophy type 2B, which has been localized to 2p13–p16 (Bashir *et al.,* 1994; Passos-Bueno *et al.,* 1995). Therefore, despite the classification of LGMD2B as a muscular dystrophy rather than a spinal atrophy, the possibility should be considered and tested that *LGMD2B* and the *wobbler* gene are homologous.

Outlook. In the near future, 40,000 ESTs will be available for the human genome (Boguski and Schuler, 1995). Thus, the chromosomal position of the *RAB1* gene and the human YACs could be used as tools for a positional candidate approach (Collins, 1995) toward the identification of the *wr* gene, similar to the successful approach to identify molecularly the gene for the murine neurological mutation *weaver* (Patil *et al.,* 1995).

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