RESEARCH

The Human Obese (OB) Gene: RNA Expression Pattern and Mapping on the Physical, Cytogenetic, and Genetic Maps of Chromosome 7

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The recently identified mouse *obese* (*ob*) gene apparently encodes a secreted protein that may function in the signaling pathway of adipose tissue. Mutations in the mouse *ob* gene are associated with the early development of gross obesity. A detailed knowledge concerning the RNA expression pattern and precise genomic location of the human homolog, the OB gene, would facilitate examination of the role of this gene in the inheritance of human obesity. Northern blot analysis revealed that OB RNA is present at a high level in adipose tissue but at much lower levels in placenta and heart. OB RNA is undetectable in a wide range of other tissues. Comparative mapping of mouse and human DNA indicated that the *ob* gene is located within a region of mouse chromosome 6 that is homologous to a portion of human chromosome 7q. We mapped the human OB gene on a yeast artificial chromosome (YAC) contig from chromosome 7q31.3 that contains 43 clones and 19 sequence-tagged sites (STSs). Among the 19 STSs are eight corresponding to microsatellite-type genetic markers, including seven $(CA)_n$ repeat-type Genethon markers. Because of their close physical proximity to the human OB gene, these eight genetic markers represent valuable tools for analyzing families with evidence of hereditary obesity and for investigating the possible association between OB mutations and human obesity.

A major focus of the ongoing human genome project is the construction of highly integrated and annotated physical, genetic, and cytogenetic maps of human chromosomes (Collins and Galas 1993; Olson 1993; Green et al. 1995b). One useful feature of such maps is the ability to localize rapidly newly discovered genes or DNA segments of interest relative to other chromosomal landmarks (e.g., genetic markers, cytogenetic bands). Establishing the physical proximity of different types of landmarks is particularly important for positional cloning projects, where all available mapping information about a critical chromosomal region (typically defined by genetic mapping studies) is valuable (Collins 1992, 1995; Ballabio 1993). It is thus not surprising that the number of disease genes isolated using a positional cloning strategy has increased dramatically since the start of the human genome project (Collins 1992, 1995; Ballabio 1993; Green et al. 1995b). However, highly annotated chromosome maps are also valuable for studies that proceed in the opposite direction; that is, from a known gene to a phenotype (such as a disease process). For example, genetic markers found to be in close physical proximity to a newly identified gene can be used to study appropriate families for evidence of genetic linkage between a particular phenotype and the gene. Thus, information derived from detailed genomic maps can provide the opportu-

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nity to study the role of a known gene in a hereditary disorder.

Recently, Zhang et al. (1994) utilized a positional cloning strategy to isolate the mouse *obese* (*ob*) gene, which when mutated results in profound obesity and type II diabetes in some inbred strains (Coleman 1978). Specifically, the *ob* gene-containing region of mouse chromosome 6 (delimited by genetic mapping) was isolated in yeast artificial chromosome (YAC) and P1 clones, and exon trapping was employed to identify candidate gene sequences. An exon exhibiting adipose tissue-specific expression was isolated and ultimately found to be part of the *ob* gene. Probes derived from the mouse *ob* gene were then used to isolate the human homolog, the OB gene. The mouse *ob* RNA is ~4.5 kb in size and encodes a 167-amino-acid open reading frame, with the predicted amino acid sequence being 84% identical in mouse and human. This protein appears to be secreted from adipose tissue and likely functions as part of a signaling pathway involved in the regulation of body fat.

The mouse *ob* gene resides on mouse chromosome 6 within a region of conserved synteny to a segment of human chromosome 7q. As part of a global effort to establish a YAC-based sequence-tagged site (STS)–content map of human chromosome 7 (Green and Green 1991; Green et al. 1991, 1994, 1995a), we sought to establish more precisely the physical location of the human homolog. Here we report the localization of the human OB gene on a YAC contig mapping to 7q31.3 that contains 43 clones and 19 STSs. Importantly, the human OB gene was found to reside in close physical proximity to a set of eight genetic markers, which can now be used to investigate a possible correlation between the gene and inherited forms of obesity. In addition, the pattern of tissue expression of OB RNA was studied, revealing that the gene is highly expressed in human adipose tissue and much less so in placenta and heart.

RESULTS AND DISCUSSION

Tissue Distribution of OB RNA

The human OB gene was isolated by Zhang et al. (1994) using a hybridization probe derived from the mouse *ob* cDNA. The sequence of the human OB gene (GenBank accession no. U18915) is highly homologous to that of the mouse gene within the amino acid-coding region, whereas the identity between the human and mouse genes is only 30% in the 3′-untranslated region. At the predicted amino acid level, there is 84% identity between the human and mouse genes (Zhang et al. 1994).

Examination of the tissue expression of the human OB gene by Northern blot analysis revealed that OB RNA is expressed at a high level in human adipose tissue and much lower levels in placenta and heart (Fig. 1). The size of the RNA (~4.5 kb) was equivalent in human and mouse as well as in each of the expressing tissues. In these studies, fivefold higher signals were seen with 10 μg of total adipose tissue RNA as with 2 μg of poly(A)$^+$ placental RNA. A fivefold lower signal was seen in poly(A)$^+$ RNA from heart compared to that from placenta. It is estimated that the level of OB RNA is ~250-fold lower in placenta than in adipose tissue. In this experiment, OB RNA was not detected in any of the other tissues analyzed, including brain, lung, liver, skeletal muscle, kidney, and pancreas. Additional experiments did not reveal OB RNA in spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocytes or in fetal brain, liver, or kidneys (data not shown). It is possible that OB is expressed at an undetectable level (by Northern blot analysis) in these latter tissues or in other tissues that were not studied. The observed pat-

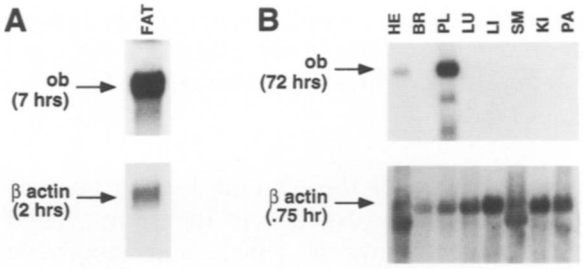

Figure 1 Northern blot analysis of human RNA. Northern blots containing 10 μg of total RNA from human adipose tissue (*A*, FAT) and 2 μg of poly(A)$^+$ RNA from other human tissues (*B*) were hybridized to human OB or human β-actin probes as indicated. An intense signal at ~4.5 kb was seen with the adipose tissue total RNA. Hybridization to the poly(A)$^+$ RNA revealed detectable signals in heart (HE) and placenta (PL), whereas OB RNA was not detected in brain (BR), lung (LU), liver (LI), skeletal muscle (SM), kidney (KI), and pancreas (PA). In each case, the length of the autoradiographic exposure is indicated. Of note, the genesis of the lower molecular weight bands seen in placental RNA (e.g., alternate splicing, RNA degradation) is not known.

tern of expression in human differs somewhat from mouse, in which *ob* RNA is detected almost exclusively in adipose tissue. The significance of expressed OB RNA in human placenta and heart is unknown, although both tissues are known to contain adipocytes. Further studies will be required to determine the expressing cell types in placenta and heart, whether the encoded protein is synthesized, and the physiological significance of these findings.

Comparative Mapping of the *ob* Gene Region in the Mouse and Human Genomes

The mouse *ob* gene is located on proximal chromosome 6 in a region homologous with a portion of human chromosome 7q. Genes within this segment include (from proximal to distal) *Met* proto-oncogene, the cystic fibrosis transmembrane conductance regulator (*Cftr*), paired box-containing gene 4 (*Pax4*), *ob*, and carboxypeptidase A (*Cpa*) (Friedman et al. 1991; Zhang et al. 1994). In mouse, genetic mapping was used to demonstrate that *Pax4* is tightly linked to *ob* (Walther et al. 1991; Zhang et al. 1994). The physical distance between *ob* and *Pax4* was found to be ~1 Mb (Zhang et al. 1994). On the basis of these comparative mapping studies, it was expected that the human OB gene would reside between PAX4 and CPA on chromosome 7q. Furthermore, because human CFTR (Heng et al. 1993) and PAX4 (Tamura et al. 1994) were mapped by fluorescence in situ hybridization (FISH) to 7q31.3 and 7q32, respectively, the most likely cytogenetic position of the human OB gene would be in the vicinity of the 7q31.3–q32 boundary.

Mapping the OB Gene on Human Chromosome 7

An STS (sWSS2619) amplifying a small segment of the 3′-untranslated region of the human OB gene was used to screen a collection of YAC clones that is highly enriched for human chromosome 7 DNA (Green et al. 1995a), and nine YACs were identified (yWSS691, yWSS1332, yWSS1998, yWSS2087, yWSS3319, yWSS3512, yWSS4875, yWSS4970, and yWSS5004). To verify that these YACs contain the authentic human OB gene, two additional experiments were performed. First, each of the YACs was tested with a second human OB-specific PCR assay, and all were found to be positive (data not shown). Second, yeast DNA from each clone was digested with *Eco*RI and analyzed by gel-transfer hybridization using a human OB cDNA-derived probe. In all instances, a single hybridizing band was seen, and this band was the same size in the YACs and a P1 clone known to contain the human OB gene (data not shown).

Using the computer program SEGMAP (Green and Green 1991; C.L. Magness and P. Green, unpubl.) and other YAC-based STS–content data that we have generated for chromosome 7 (Green et al. 1991, 1994, 1995a), the human OB gene was found to reside within the YAC contig depicted in Figure 2. Specifically, this contig consists of 43 overlapping YACs and 19 uniquely ordered STSs. Details about each of the 19 STSs are provided in Table 1. In addition to the OB-specific STS, the contig also contains an STS (sWSS808) specific for the human PAX4 gene (Tamura et al. 1994; Stapleton et al. 1993), seven STSs derived from chromosome 7-specific YACs, two STSs derived from chromosome 7-specific λ clones, and, importantly, eight microsatellite-specific STSs. Additional details about these eight genetic markers, including sequences of the primers used for genotype analysis, are provided in Table 2. Of note, there is redundant YAC-based connectivity throughout the contig (i.e., there are two or more YACs connecting each adjacent pair of STSs), lending strong support for the relative order of STSs shown in Figure 2.

As depicted in Figure 2, the predicted orientation of the human OB-containing YAC contig is such that sWSS1734 is the centromeric-most STS (i.e., closest to CFTR), whereas sWSS2367 is the telomeric-most STS (i.e., closest to CPA). This orientation is predominantly based on comparative mapping data, which places *Pax4* proximal and *ob* distal within the syntenic block present in mouse and human DNA (Zhang et al. 1994). The OB gene maps near the telomeric end of the contig, based on the placement of the OB-specific STS (sWSS2619).

Although the contig shown in Figure 2 was deduced by SEGMAP without consideration of YAC sizes (thereby displaying STSs equidistant from one another), a similar analysis of the data by SEGMAP that accounted for YAC sizes indicated that the total size of the region covered by the contig is just over 2 Mb (data not shown). Thus, while all eight of the microsatellite-specific STSs (Table 2) are contained within a genomic interval spanning ~2 Mb, the three closest to the telomeric end of the contig (sWSS1392,

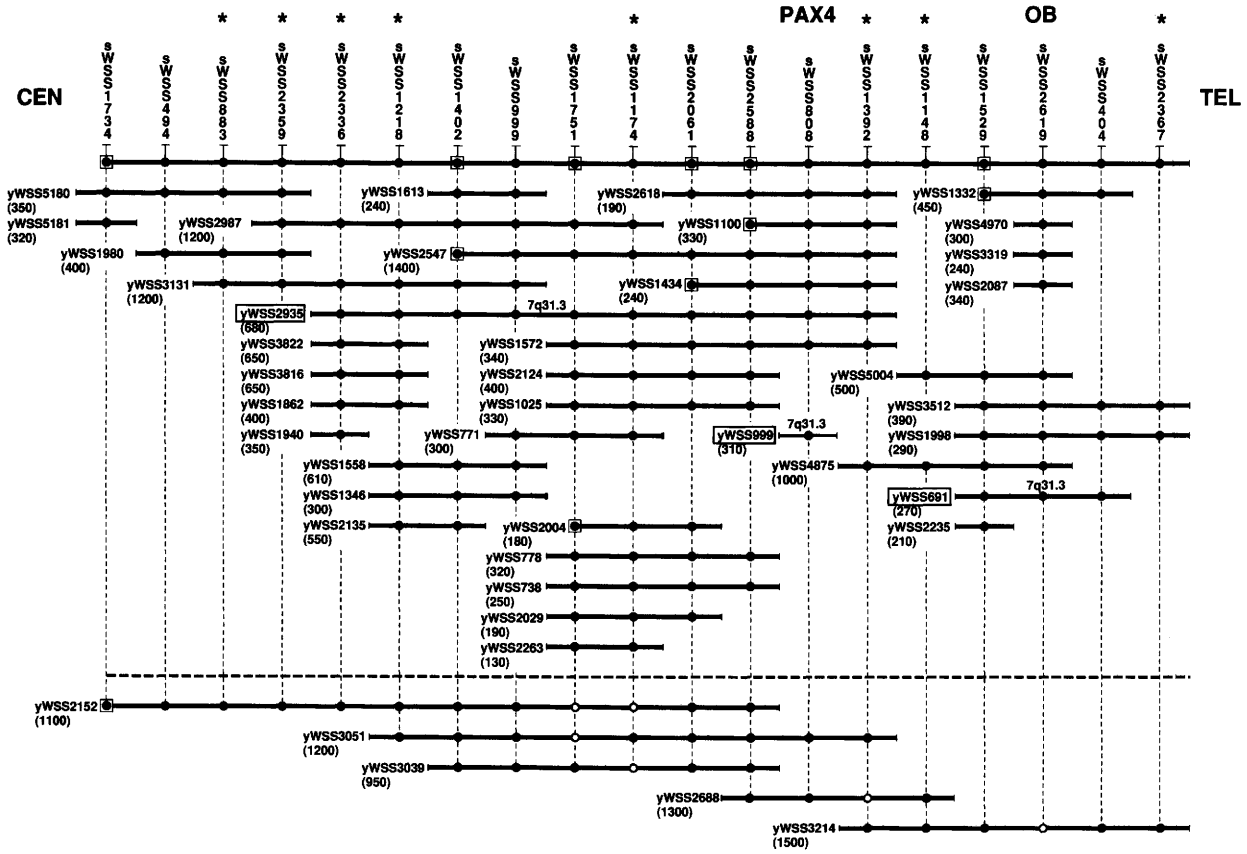

Figure 2 YAC contig containing the human OB gene and eight microsatellite markers. The YAC-based STS–content map of the region of chromosome 7 containing the human OB gene is depicted, as deduced by SEGMAP/version 3.29 (Green and Green, 1991; C.L. Magness and P. Green, unpubl.). The 19 uniquely ordered STSs (see Table 1) are listed along the *top*; the eight microsatellite-specific STSs are indicated with stars (see Table 2). Also indicated are the STSs corresponding to the PAX4 and OB genes, as well as the predicted positions of the centromere (CEN) and 7q telomere (TEL) relative to the contig. Each of the 43 YAC clones is depicted by a horizontal bar, with its name given to the *left* and estimated YAC size (in kb, measured by pulsed-field gel electrophoresis) provided in parenthesis. The presence of an STS in a YAC is indicated (•) at the appropriate position. When an STS corresponds to the insert end of a YAC, a square is placed around the corresponding circle, both along the *top* (near the STS name) and at the end of the YAC from which it was derived. For the five YACs at the *bottom* (below the horizontal broken line), one or more STS expected to be present (based on the established STS order) was not detected (as assessed by testing the individual YACs with the corresponding STS-specific PCR assays at least twice), and these are depicted (○) at the appropriate positions. Most of the YACs were isolated from a human–hamster hybrid cell-derived library (Green et al. 1995a), with their original names as indicated. The remaining YACs were isolated from total human genomic libraries, and their original library locations are provided in Table 3. Boxes are placed around the names of the three YACs (yWSS691, yWSS999, and yWSS2935) that were found by FISH analysis to map to 7q31.3. The contig is displayed in its "uncomputed" form, where YAC sizes are not used to estimate clone overlaps or STS spacing, and all of the STSs are therefore spaced in an equidistant fashion. In the computed form, where YAC sizes are used to estimate the relative distance separating each pair of adjacent STSs as well as the extent of clone overlaps, the total YAC contig appears to span just over 2 Mb.

sWSS1148, and sWSS2367) are particularly close to the OB gene itself (perhaps within an interval as small as ~500 kb). All three of the latter STSs are present in at least one of the human OB-containing YACs. Of note, the interval between human PAX4 (sWSS808) and OB (sWSS2619) is estimated to be ~400 kb, whereas this region was predicted to span ~1 Mb in mouse (Zhang et al. 1994). Finally, three of the YACs within the contig (yWSS691, yWSS999, and yWSS2935) have also been analyzed by FISH, and each was found to hybridize exclusively to 7q31.3 (T. Feather-

Table 1. STSs in the YAC contig containing the human OB gene

STS Name	Alias	Locus	Source	PCR Primers	Size (bp)	GDB ID No.
sWSS1734		D7S2185	YAC End	CAAGACAAATGAGATAAGG AGAGTTACAGCTTTACAG	72	G00-455-235
sWSS494		D7S2016	Lambda Clone	CTAAACACCTTTCCATTCC TTATATTCACTTTTCCCCTCTC	112	G00-334-404
sWSS883	UT528	D7S1498	Genetic Marker	TGCAGTAAGCTGTGATTGAG GTGCAGCTTTAATTGTGAGC	490	G00-455-262
sWSS2359	AFMa065zg9	D7S1873	Genetic Marker	AGTGTTGTGTTTCTCCTG AAAGGGGATGTGATAAGTG	142	G00-455-247
sWSS2336	AFMa125wh1	D7S1874	Genetic Marker	GGTGTTACGTTTAGTTAC GGAATAATGAGAGAAGATTG	112	G00-455-244
sWSS1218	AFM309yf1	D7S680	Genetic Marker	GCTCAACTGACAGAAAAC GACTATGTAAAAGAAATGCC	154	G00-307-733
sWSS1402		D7S1916	YAC End	AAAGGGCTTCTAATCTAC CCTTCCAACTTCTTTGAC	137	G00-344-044
sWSS999		D7S1674	YAC Insert	TAAACCCCCTTTCTGTTC TTGCATAATAGTCACACCC	105	G00-334-839
sWSS1751		D7S2186	YAC End	CCAAAATCAGAATTGTCAGAAG AAACCGAAGTTCAGATACAG	186	G00-455-238
sWSS1174	AFM218xf10	D7S514	Genetic Marker	AATATCTGACATTGGCAC TTAGACCTGAGAAAAGAG	144	G00-307-700
sWSS2061		D7S2184	YAC End	GTTGCACAATACAAAATCC CTTCCATTAGTGTCTTATAG	200	G00-455-241
sWSS2588		D7S2187	YAC End	ATCACTACACACCTAATC CCATTCTACATTTCCACC	117	G00-455-253
sWSS808	PAX4	PAX4	Gene	GGCTGTGTGAGCAAGATCCTAGGA TTGCCAGGCAAAGAGGGCTGGAC	153	G00-455-259
sWSS1392	AFM206xc1	D7S635	Genetic Marker	CTCAGGTATGTCTTTATC TGTCTCTGCATTCTTTTC	75	G00-307-815
sWSS1148	AFM199xh12	D7S504	Genetic Marker	GACACATACAAACACAAG ATTGAGTTGAGTGTAGTAG	60	G00-307-652
sWSS1529		D7S1943	YAC End	CAGGGATTTCTAATTGTC AAAAGATGGAGGCTTTTG	116	G00-334-119
sWSS2619	OB	OB	Gene	CGTTAAGGGAAGGAACTCTGG TGGCTTAGAGGAGTCAGGGA	106	G00-455-256
sWSS404		D7S1956	Lambda Clone	ACCAGGGTCAATACAAAG TAATGTGTCCTTCTTGCC	122	G00-334-251
sWSS2367	AFMa345wc9	D7S1875	Genetic Marker	CAATCCTGGCTTCATTTG AAGGTGGGTAGGATGCTA	81	G00-455-250

The 19 chromosome 7-specific STSs mapped to the YAC contig containing the human OB gene (Fig. 2) are listed. In each case, the designated sWSS name, relevant alias, GDB-assigned locus name, STS source, PCR primer sequences, STS size, and GDB identification (ID) number are indicated. The sources of STSs are as follows: (YAC End) isolated insert end of a YAC (Green 1993); (λ Clone) random chromosome 7-specific λ clone (Green et al. 1991; Green 1993); (Genetic Marker) microsatellite marker (Green et al. 1994; see Table 2); (YAC Insert) random segment from YAC insert; (Gene) gene-specific STS. Note that for some genetic marker-specific STSs, the PCR primers used for identifying YACs (listed here) are different from those used for performing genotype analysis (Table 2), because the detection of YACs containing a genetic marker does not require amplification of the polymorphic tract itself. All of the indicated PCR assays utilized an annealing temperature of 55°C, except for sWSS494, sWSS883, sWSS1529, and sWSS2619 (which used 50°C), sWSS999 and sWSS1174 (which used 60°C), and sWSS808 (which used 65°C). Additional details regarding the STS-specific PCR assays are available in GDB.

Table 2. Microsatellite markers in the YAC contig containing the human OB gene

Marker Name	Type	Locus	Primers	GDB ID No.
UT528	Tetra.	D7S1498	TGCAGTAAGCTGTGATTGAG GTGCAGCTTTAATTGTGAGC	G00-312-446
AFMa065zg9	$(CA)_n$	D7S1873	AGCTTCAAGACTTTNAGCCT GGTCAGCAGCACTGTGATT	G00-437-253
AFMa125wh1	$(CA)_n$	D7S1874	TCACCTTGAGATTCCATCC AACACCGTGGTCTTATCAAA	G00-437-263
AFM309yf10	$(CA)_n$	D7S680	CATCCAAGTTGGCAGTTTTT AGATGCTGAATTCCCAGACA	G00-200-283
AFM218xf10	$(CA)_n$	D7S514	TGGGCAACACAGCAAA TGCAGTTAGTGCCAATGTCA	G00-188-404
AFM206xc1	$(CA)_n$	D7S635	CCAGGCCATGTGGAAC AGTTCTTGGCTTGCGTCAGT	G00-199-240
AFM199xh12	$(CA)_n$	D7S504	TCTGATTGCTGGCTGC GCGCGTGTGTATGTGAG	G00-188-280
AFMa345wc9	$(CA)_n$	D7S1875	AGCTCTTGGCAAACTCACAT GCCTAAGGGAATGAGACACA	G00-437-259

The eight microsatellite markers mapped to the YAC contig containing the human OB gene (Fig. 2) are listed. In each case, the marker name (indicated as the alias in Table 1), type of microsatellite motif [tetranucleotide (Tetra.) repeat or $(CA)_n$ repeat], GDB-assigned locus name, primer sequences utilized for PCR-based genotype analysis, and GDB identification (ID) number are indicated. Additional details regarding the PCR assays and the polymorphisms are available in GDB.

stone and E.D. Green, unpubl.). One of these YACs (yWSS691) contains the OB-specific STS, whereas the other two clones contain the PAX4-specific STS. The latter results are generally consistent with the previous cytogenetic assignment of human PAX4 to 7q32 (Tamura et al. 1994). On the basis of these data, the human OB gene can be assigned to cytogenetic band 7q31.3.

SUMMARY

OB RNA is expressed at high levels in human adipose tissue and at substantially lower levels in placenta and heart. The human OB gene maps to a large YAC contig derived from chromosome 7q31.3. In addition to confirming the relative location of the gene based on mouse–human comparative mapping, this study has identified eight established microsatellite markers in close physical proximity to the human OB gene. Because mutations in mouse *ob* can result in a syndrome that closely resembles morbid obesity in humans, these genetic markers represent important tools for studying the possible role of the OB gene in inherited forms of human obesity.

METHODS

Northern Blot Analysis

Total RNA was prepared from adipose tissue using the method of Chirgwin et al. (1979). Northern blots, radiolabeling, and hybridizations were performed as described (Zhang et al. 1994). Northern blots of poly(A)$^+$ RNA (human MTN, human MTN II, and human fetal MTN II) were purchased from CLONTECH (Palo Alto, CA), as were PCR primers used to generate the radiolabeled human actin probe.

STS Development

STS-specific PCR assays were developed and optimized essentially as described (Green and Green 1991; Green et al. 1991, 1994; Green 1993). Each STS is named using the prefix "sWSS" followed by a unique number. Details about the 19 STSs reported here are provided in Table 1, with additional information (e.g., PCR reaction conditions, complete DNA sequence) available in GenBank and/or the Genome Data Base (GDB). For the microsatellite-specific STSs, the oligonucleotide primers used in the PCR assays (Table 1) corresponded either to those employed for genotype analysis (Table 2; Gyapay et al. 1994) or those designed [most often with the computer program OSP (Hillier and Green 1991)] using the DNA sequence available in GenBank or provided by J. Weissenbach.

The human OB-specific STS (sWSS2619) was designed using DNA sequence obtained from the 3′-untranslated region of the cDNA. The human PAX4-specific STS (sWSS808) was developed using the following strategy. Oligonucleotide primers specific for the mouse *Pax4* gene [GGCTGTGTGAGCAAGATCCTAGGA and GGGAGCCTTGTCCTGGGTACAAAG (Walther et al. 1991)] were used to amplify a 204-bp fragment from human genomic DNA (which was the same size product as that generated from mouse genomic DNA). This PCR assay was not suitable for identifying corresponding YACs, as a similarly sized (200-bp) product was also amplified from yeast DNA. However, DNA sequence analysis of the PCR product generated from human DNA revealed substitutions at 20 positions among the 156 bases analyzed (data not shown). Using this human-specific sequence, a new primer (TTGCCAGGCAAAGAGGGCTGGAC) was designed and used with the first of the above mouse *Pax4*-specific primers (see Table 1). The resulting human PAX4-specific PCR assay did not amplify

Table 3. Original well locations of YACs derived from total human genomic libraries

yWSS Name	Library	Location
yWSS2547	CEPH	742E10
yWSS2618	CEPH	764E01
yWSS2688	CEPH	773H12
yWSS2935	CEPH	799C08
yWSS2987	CEPH	805C08
yWSS3039	CEPH	812E03
yWSS3051	CEPH	813F10
yWSS3131	CEPH	830D04
yWSS3214	CEPH	848H08
yWSS3319	CEPH	875F09
yWSS4875	CEPH	928C01
yWSS4970	CEPH	943F10
yWSS5004	CEPH	950B10
yWSS5180	ICI	92H3
yWSS5181	ICI	308F10

Most of the YACs depicted in Fig. 2 were isolated from a human-hamster hybrid cell-derived library (Green et al. 1995a), with their original names indicated in the contig. A subset of the YACs depicted in Fig. 2 were isolated from total human genomic libraries constructed at CEPH (Albertsen et al. 1990; Dausset et al. 1992) or ICI (Anand et al. 1989, 1990). To facilitate cross-correlation of these latter YACs, each yWSS name is listed, along with the original library and precise well location from which the corresponding clone was isolated.

a significant product from yeast DNA and was thus used for identifying corresponding YACs.

Identification of YACs by PCR-based Screening

Most of the YACs reported here were derived from a collection of clones highly enriched for human chromosome 7 DNA [the "chromosome 7 YAC resource" (Green et al. 1995a)] using a PCR-based screening strategy (Green and Olson 1990; Green et al. 1995). In a few cases, clones were isolated by PCR-based screening (Green and Olson 1990) of available total human genomic YAC libraries constructed at Centre d'Etude du Polymorphisme Humain (CEPH) (Albertsen et al. 1990; Dausset et al. 1992) or ICI (Anand et al. 1989, 1990). Each YAC is named using the prefix "yWSS" followed by a unique number. Of note, the original library positions of all YACs derived from total human genomic libraries are listed in Table 3.

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REFERENCES

Albertsen, H.M., H. Abderrahim, H.M. Cann, J. Dausset, D. Le Paslier, and D. Cohen. 1990. Construction and characterization of a yeast artificial chromosome library containing seven haploid genome equivalents. *Proc. Natl. Acad. Sci.* **87:** 4256–4260.

Anand, R., A. Villasante, and C. Tyler-Smith. 1989. Construction of yeast artificial chromosome libraries with large inserts using fractionation by pulsed-field gel electrophoresis. *Nucleic Acids Res.* **17:** 3425–3433.

Anand, R., J.H. Riley, R. Butler, J.C. Smith, and A.F. Markham. 1990. A 3.5 genome equivalent multi access YAC library: construction, characterisation, screening and storage. *Nucleic Acids Res.* **18:** 1951–1956.

Ballabio, A. 1993. The rise and fall of positional cloning? *Nature Genet.* **3:** 277–279.

Chirgwin, J.J., A.E. Przbyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18:** 5294–5299.

Coleman, D.L. 1978. Obese and diabetes: Two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* **14:** 141–148.

Collins, F.S. 1992. Positional cloning: let's not call it reverse anymore. *Nature Genet.* **1:** 3–6.

Collins, F.S. 1995. Positional cloning moves from perditional to traditional. *Nature Genet.* **9:** 347–350.

Collins, F. and D. Galas. 1993. A new five-year plan for the U.S. human genome project. *Science* **262:** 43–46.

Dausset, J., P. Ougen, H. Abderrahim, A. Billault, J.-L. Sambucy, D. Cohen, and D. Le Paslier. 1992. The CEPH YAC library. *Behring Inst. Mitt.* **91:** 13–20.

Friedman, J.M., R.L. Leibel, D.S. Siegel, J. Walsh, and N. Bahary. 1991. Molecular mapping of the mouse *ob* mutation. *Genomics* **11:** 1054–1062.

Green, E.D. 1993. Physical mapping of human chromosomes: generation of chromosome-specific sequence-tagged sites. In *Methods in molecular genetics*

(Vol. 1): Gene and chromosome analysis (Part A) (ed. K.W. Adolph), pp. 192–210. Academic Press, San Diego, CA.

Green, E.D. and P. Green. 1991. Sequence-tagged site (STS) content mapping of human chromosomes: theoretical considerations and early experiences. *PCR Methods Applic.* **1:** 77–90.

Green, E.D. and M.V. Olson. 1990. Systematic screening of yeast artificial-chromosome libraries by use of the polymerase chain reaction. *Proc. Natl. Acad. Sci.* **87:** 1213–1217.

Green, E.D., R.M. Mohr, J.R. Idol, M. Jones, J.M. Buckingham, L.L. Deaven, R.K. Moyzis, and M.V. Olson. 1991. Systematic generation of sequence-tagged sites for physical mapping of human chromosomes: Application to the mapping of human chromosome 7 using yeast artificial chromosomes. *Genomics* **11:** 548–564.

Green, E.D., J.R. Idol, R.M. Mohr-Tidwell, V.V. Braden, D.C. Peluso, R.S. Fulton, H.F. Massa, C.L. Magness, A.M. Wilson, J. Kimura, J. Weissenbach, and B.J. Trask. 1994. Integration of physical, genetic and cytogenetic maps of human chromosome 7: Isolation and analysis of yeast artificial chromosome clones for 117 mapped genetic markers. *Hum. Mol. Genet.* **3:** 489–501.

Green, E.D., V.V. Braden, R.S. Fulton, R. Lim, M.S. Ueltzen, D.C. Peluso, R.M. Mohr-Tidwell, J.R. Idol, L.M. Smith, I. Chumakov, D. Le Paslier, D. Cohen, T. Featherstone, and P. Green. 1995a. A human chromosome 7 yeast artificial chromosome (YAC) resource: Construction, characterization, and screening. *Genomics* **25:** 170–183.

Green, E.D., D.R. Cox, and R.M. Myers. 1995b. The human genome project and its impact on the study of human disease. In *The metabolic and molecular bases of inherited disease* (ed. C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle), pp. 401–436. McGraw Hill, New York.

Gyapay, G., J. Morissette, A. Vignal, C. Dib, C. Fizames, P. Millasseau, S. Marc, G. Bernardi, M. Lathrop, and J. Weissenbach. 1994. The 1993-94 Genethon human genetic linkage map. *Nature Genet.* **7:** 246–249.

Heng, H.H.Q., X.-M. Shi, and L.-C. Tsui. 1993. Fluorescence in situ hybridization mapping of the cystic fibrosis transmembrane conductance regulator (CFTR) gene to 7q31.3. *Cytogenet. Cell Genet.* **62:** 108–109.

Hillier, L. and P. Green. 1991. OSP: A computer program for choosing PCR and DNA sequencing primers. *PCR Methods Applic.* **1:** 124–128.

Olson, M.V. 1993. The human genome project. *Proc. Natl. Acad. Sci.* **90:** 4338–4344.

Stapleton, P., A. Weith, P. Urbanek, Z. Kozmik, and M. Busslinger. 1993. Chromosomal localization of seven *PAX* genes and cloning of a novel family member, *PAX-9*. *Nature Genet.* **3:** 292–298.

Tamura, T., Y. Izumikawa, T. Kishino, H. Soejima, Y. Jinno, and N. Niikawa. 1994. Assignment of the human PAX4 gene to chromosome band 7q32 by fluorescence *in situ* hybridization. *Cytogenet. Cell Genet.* **66:** 132–134.

Walther, C., J.-L. Guenet, D. Simon, U. Deutsch, B. Jostes, M.D. Goulding, D. Plachov, R. Balling, and P. Gruss. 1991. Pax: A murine multigene family of paired box-containing genes. *Genomics* **11:** 424–434.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J.M. Friedman. 1994. Positional cloning of the mouse *obese* gene and its human homologue. *Nature* **372:** 425–432.

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