Supporting Online Material

Materials and methods

Transgenic mice

YAC-290 was generated by truncating the YAC-460 construct with the fragmentation vector pU-V22 (1). The URA3 gene in the arm was replaced with the LYS2 gene using pLT (1). To make IRES-WGA, the gap-EGFP portion of IRES-gap-EGFP (1) was replaced with a coding region of the truncated WGA (2). To make IRES-tau-DsRed, IRES-tau-ECFP and IRES-tau-EYFP, the EGFP portion of IRES-tau-EGFP was replaced with DsRed, ECFP and EYFP (Clontech), respectively. These fragments for tagging were inserted immediate downstream from the stop codons of the MOR10, MOR83 and MOR29 genes in the YAC-290 construct. The H region flanked by two ScaI sites in the MunI-SfiI fragment of YAC-290 was deleted, producing the MSdH fragment. The URA3 gene and the MSdH fragment were inserted into pBluescript vector, generating pUdH. The 2.1 kb H region flanked by two ScaI sites was removed from YAC-290 by yeast homologous recombination (3) with pUdH, generating the YAC-290dH. Plasmid pU-H50L was made by inserting the H-containing ScaI-ScaI fragment into the SpeI site of pU-50L (1). Plasmid pU-3k was a derivative of the pBluescript containing the Tetrahymena telomere sequence, URA3 gene, and a BamHI-SphI fragment isolated from the upstream region of the MOR28 promoter. Plasmid pU-H3k was made by inserting the 2.1 kb H
region fragment into the 3’ side of the \textit{URA3} gene in pU-3k. YAC constructs, 180, H180, 140, and H140 were generated by truncating YAC-290 with pU-50L, pU-H50L, pU-3k, and pU-H3k, respectively. To make the \textit{MOR28} minigene, the BamHI-BamHI fragment containing \textit{MOR28} was isolated from the C57BL/6 mouse BAC clone, RPCI-23-339P24 (CHORI), and was subcloned into pBluescript. The \textit{IRES-tau-ECFP} or \textit{IRES-tau-EYFP} fragment was inserted into the SpeI site downstream from the \textit{MOR28} coding region, generating the \textit{MOR28} minigene. The \textit{H-MOR28} construct was made by joining the H-containing ScaI-ScaI fragment to the 5’ end of the \textit{MOR28} minigene. The YAC-290G construct was generated by truncating YAC-460G \textit{(1)} with a fragmentation vector pU-V22, and by replacing the \textit{URA3} gene in the arm with the \textit{LYS2} gene using the pLT plasmid. To create the YAC-290GdC, a 1 kb region upstream of the \textit{MOR28} start codon and a 0.2 kb region downstream of the \textit{MOR28} stop codon were subcloned into pGEM-T (Promega) by recombinant PCR. DNA fragments were ligated via EcoRI sites created immediately upstream of the start codon and immediately downstream of the stop codon, generating p28dC. The \textit{IRES-tau} fragment isolated from the \textit{IRES-tau-EGFP} tag \textit{(1)} was inserted into the SpeI site of p28dC, and the \textit{URA3} gene from YIp5 was added to the vector, generating the pU-28dCIT. The \textit{MOR28} coding region was deleted from the YAC-290G using the pU-28dCIT by yeast homologous recombination \textit{(3)} to generate the YAC-290GdC. Three lines of transgenic mice were obtained for both YAC-290G and YAC-290GdC.
constructs. Copy numbers of the transgenic constructs in each line were quantified by Southern hybridization: YAC-290G line 1 (4 copies), line 2 (2 copies) and line 3 (6 copies); YAC-290GdC line 1 (2 copies), line 2 (2 copies) and line 3 (4 copies). For microinjection, the MOR28 or H-MOR28 minigene was cleaved from the plasmid with BssHII. The YAC DNA was extracted and injected as previously described (4).

**OR gene probes**

To collect the OR genes whose expression areas in the OE are overlapped with that of the MOR28, partial coding fragments of 400-600 bp isolated from 253 different OR genes were amplified by PCR from the C57BL/6 genomic DNA. The PCR products were subcloned into pGEM-T (Promega) and used as templates to make RNA probes. Expression patterns of 212 OR genes were examined by *in situ* hybridization of the OE sections. This collection of 212 OR genes includes at least one member of each subfamily among 186 different class II subfamilies (5). Once we found the OR genes whose expression areas in the OE overlapped with that of the MOR28, we examined the remaining members within their subfamilies for their expression in the OE. In this way, a total 253 OR genes were analyzed for the expression areas in the OE. The OR genes with overlapping expression areas with the MOR28 were examined for the co-expression with the del-MOR28 transgene. Each mixed probe shown in Fig. 4 contained the following OR gene probes: 230 mix (MOR230-1, 230-4, 230-5),
231 mix (231-1, 232-1, 234-1, 235-1, 236-1), 233 mix (233-2, 233-6, 233-7, 233-10, 233-16), 246 mix (246-2, 246-4, 246-6), 248 mix (248-2, 248-11), 271 mix (271-1, 272-2, 273-1, 274-1), 286 mix (286-1, 286-2, 286-3), and other (184-1, 206-2, 221-1, 225-1, 226-1). For 244-2 (MOR10) and pseudo OR genes, DNA fragments of 400-600 bp were amplified from the 3’ UTR by PCR from the C57BL/6 genomic DNA. They were then subcloned into pGEM-T (Promega) and used as templates to make RNA probes.

**Histochemistry**

Mice were perfused intracardially with 4% paraformaldehyde (PFA) in PBS. The OE and OB samples were dissected and fixed overnight with 4% PFA in PBS. The OE was decalcified in 0.5M EDTA in PBS overnight. The tissues were frozen in OCT (Tissue-Tek) and coronal cryostat sections (10-16 µm each) were made. Preparation of the digoxigenin-labeled RNA probes (Hoffmann-La Roche) and in situ hybridization were performed as described (6). For fluorescent in situ hybridization, the hybridized OE sections were treated with the anti-DIG polyclonal antibodies (Hoffmann-La Roche), and then incubated with biotin-conjugated anti-sheep IgG antibodies (Chemicon International) (1:200) in PBS for 1hr at room temperature. After washing, the samples were reacted with the HRP-conjugated streptavidin (PerkinElmer) (1:100) in PBS for 30min. Signals were visualized by incubating the samples with TSA-Cy3 for 10min using Tyramide signal amplification kit (PerkinElmer) according to the
manufacturer’s instructions. For immunostaining, the rabbit antibodies against EGFP (Clontech) (1:1,000) and FITC-conjugated or Cy3-conjugated secondary anti-rabbit IgG antibodies (Chemicon International) (1:500) were applied. Immunostaining of WGA was performed as described (2). Whole nuclei were counterstained with DAPI (Hoffmann-La Roche) for the OB sections.

**Fluorescent photos**

EGFP on the whole mount OB was imaged by a fluorescence microscope, Model SZX12 (Olympus), and photographed with a cooled CCD camera, Model C4742-95-12ERG (Hamamatsu Photonics). Fluorescent signals of Cy3 and DAPI on the OB sections were captured with a fluorescence microscope, Model IX70 (Olympus), coupled to a cooled CCD camera, C4742-95-12ERG (Hamamatsu Photonics). Fluorescent signals of EGFP, Cy3 and FITC on the OE sections were captured with a confocal microscope, Model BX61 (Olympus).

**Supporting references and notes**