

CLONING OF NOVEL SEVEN TRANSMEMBRANE-DOMAIN RECEPTORS FROM RAT OLFACTORY NEUROEPITHELIUM

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Abstract: The olfactory system of vertebrates is able to translate olfactory stimuli into complex sensory information. The olfactory transduction is initiated by the binding of an odorant ligand to a protein receptor on the apical surface of olfactory sensory neurons. The olfactory receptors are 7-transmembrane-domain proteins belonging to a large multigene family. The rat OR gene family has been estimated to include approximately 500-1000 genes.

In initial experiments, we designed a series of degenerate oligonucleotides that could anneal to conserved regions of 96 members of the OR gene family. The synthetic oligonucleotides were used in PCR reactions to amplify homologous sequences in cDNA synthesized by reverse transcription of polyA⁺ RNA purified from rat olfactory neuroepithelium. The amplification products were within the size range expected for this family of receptors. The PCR products were subsequently inserted in a plasmid vector and individual clones were isolated. Sequence analysis of 48 random clones revealed that all were new members of the OR family and were distributed broadly across a similarity dendrogram.

Two amplification products showing the highest sequence divergence were used as molecular probes to screen a cDNA library constructed from rat olfactory neuroepithelium polyA⁺ RNA and cloned in a phage vector. Three full-length cDNA clones were isolated by screening 3.6×10^5 recombinant phages. Sequence analysis of the cloned cDNAs showed that each clone is a new member of the OR family. In situ hybridization experiments indicate that these OR genes are expressed selectively in the sensory neurons of the rat olfactory neuroepithelium.

Keywords: olfactory system, receptors