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The ocular albinism type 1 gene product, OA1, spans intracellular membranes 7 times

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Abstract

OA1 (GPR143) is a pigment cell-specific intracellular glycoprotein consisting of 404 amino acid residues that is mutated in patients with ocular albinism type 1, the most common form of ocular albinism. While its cellular localization is suggested to be endolysosomal and melanosomal, the physiological function of OA1 is currently unclear. Recent reports predicted that OA1 functions as a G protein coupled receptor (GPCR) based on its weak amino acid sequence similarity to known GPCRs, and on demonstration of GPCR activity in OA1 mislocalized to the plasma membrane. Because mislocalization of proteins is often caused by or induces defects in their proper folding/assembly, the significance of these studies remains unclear. A characteristic feature of GPCRs is a seven transmembrane domain structure. We analyzed the membrane topology of OA1 properly localized to intracellular lysosomal organelles in COS-1 cells. To accomplish this analysis, we established experimental conditions that allowed selective permeabilization of the plasma membrane while leaving endolysosomal membranes intact. Domains were mapped by the insertion of a hemagglutinin (HA) tag into the predicted cytosolic/luminal regions of OA1 molecule and the accessibility of tag to HA antibody was determined by immunofluorescence. HA-tagged lysosome associated membrane protein 1 (LAMP1), a type I membrane protein, was employed as a reporter for selective permeabilization of the plasma membrane. Our results show experimentally that the C-terminus of OA1 is directed to the cytoplasm and that the protein spans the intracellular membrane 7 times. Thus, OA1, properly localized intracellularly, is a 7 transmembrane domain integral membrane protein consistent with its putative role as an intracellular GPCR.

Keywords: membrane topology; lysosome; cellular localization; ocular albinism type 1; melanosome; G protein coupled receptor; selective membrane permeabilization

1. Introduction

Ocular albinism type 1 (OA1) is the most common form of ocular albinism (van Dorp, 1987; Shen et al., 2001b). OA1 is inherited in an X-linked recessive fashion and is characterized by a severe reduction of visual acuity, refractive errors, nystagmus, iris translucency, fundus hypopigmentation, foveal hypoplasia, and loss of stereoscopic vision due to misrouting of the optic fibers at the optic chiasm (Charles et al., 1993; Creel et al., 1990; Kriss et al., 1992; King et al., 1995). Microscopic examination of eye and skin tissue from OA1 patients reveals the presence of macromelanosomes or melanin macroglobules (MMGs) (Garner and Jay, 1980; Wong et al., 1983).

The human OA1 gene was identified by positional cloning (Bassi et al., 1995). The OA1 gene product encodes a pigment cell-specific glycoprotein consisting of 404 amino acid residues. While its cellular localization is suggested to be endolysosomal and melanosomal, the physiological function is currently

Abbreviations: OA1, ocular albinism type 1; GPCR, G protein coupled receptor; HA, influenza hemagglutinin epitope; LAMP, lysosome associated membrane protein; MMGs, melanin macroglobules; TM, transmembrane; DEME, Dulbecco's modified Eagle's Medium; PAGE, polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; HRP, horseradish peroxidase; PBS, phosphate buffered saline; GFP, green fluorescent protein.

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unknown (Schiaffino et al., 1996; Samaraweera et al., 2001). While early analysis suggested that the protein had 6 transmembrane domains (Newton et al., 1996), homology analysis indicates a weak similarity to the G protein coupled receptor superfamily (GPCR; type A, B, and E) (Schiaffino et al., 1999; Schiaffino and Tacchetti, 2005). When expressed in non-melanocytic cells such as COS-1, the mouse ortholog Oa1 displays a vesicular distribution and colocalizes with the late endosomal/lysosomal markers, LAMP2 and CD63 (Shen et al., 2001a).

Recent results obtained with several experimental systems suggest that OA1 can function as a GPCR (Schiaffino et al., 1999; Staleva and Orlow, 2006; Innamorati et al., 2006). A study employing *Saccharomyces cerevisiae* as a model system (Staleva and Orlow, 2006) revealed that: (1) mouse Oa1 localizes to the prevacuolar compartment (functionally equivalent to the mammalian late endosome); (2) Oa1 can function as a GPCR in a yeast-based GPCR signaling assay; and (3) candidate ligands for Oa1 exist in melanocyte extracts. Studies involving purposeful mislocalization of human OA1 to the plasma membrane in mammalian cells (Schiaffino et al., 1999; Innamorati et al., 2006) indicate that the mislocalized OA1 can activate heterotrimeric G proteins and may be associated with arrestin. Both experimental systems suggest that OA1 functions as a GPCR in a similar fashion to GPCRs known to be localized at the plasma membrane.

Given that OA1 is a GPCR, it is expected to span integral membranes 7 times, with its C-terminus oriented to the cytoplasm. However, OA1 was originally predicted to have 6 transmembrane (6TM) regions (Bassi et al., 1995) based on Kyte-Doolittle plots. The SOSUI algorithm (Hirokawa et al., 1998), which classifies integral membrane proteins, also predicts 6TM regions for OA1. However, biochemical studies regarding the mechanism of insertion of transmembrane domains into the lipid bilayer have shown that hydrophobicity is not the unique crucial factor for determining the membrane topology of integral membrane proteins (Ota et al., 1998a,b).

Employing *Saccharomyces cerevisiae* as a model system, Staleva and Orlow demonstrated the existence of candidate ligands for OA1 in melanocytes (Staleva and Orlow, 2006). However a specific ligand has not yet been identified. If OA1 is truly an intracellular 7TM GPCR, then its ligand should exist with the lumen of intracellular organelles such as endolysosomes or melanosomes. As a prelude to embarking upon a major search for ligands, we deemed it critical to establish the membrane topology of intracellular OA1.

In addition, two separate dileucine motifs that purportedly target the OA1 molecule to lysosomal organelles impinge upon one of the predicted TM regions suggesting two possible orientations for OA1. To definitively establish the transmembrane orientation of OA1, we developed methodologies allowing selective permeabilization of plasma membrane without disruption of intracellular endolysosomal membranes. We adapted a technique involving cold osmotic shock to selectively permeabilize the plasma membrane (Eckhardt et al., 1999) of cells and then determined the orientation of an HA-tag inserted at various points in the predicted hydrophilic domains of the OA1 molecule. Our study demonstrates that OA1 has 7TM regions and that its C-terminus is found in the cytosol, consistent with the concept that OA1 may function as an intracellular GPCR localized to endolysosomal organelles.

2. Materials and methods

2.1. Antibodies

A BD Living Colors A.v. peptide antibody, directed against GFP (rabbit polyclonal), was obtained from BD Biosciences (Palo Alto, CA). Monoclonal antibody 16B12, directed against the hemagglutinin (HA) epitope YPYDVPDYA, was obtained from COVANCE (Richmond, CA), and monoclonal antibody H5C6, directed against CD63, was obtained from Developmental Studies Hybridoma Bank (Ames, IA). HRP-conjugated goat anti-rabbit IgG was from BIO-RAD (Hercules, CA), and Texas Red conjugated goat anti-mouse IgG from Jackson ImmunoResearch (West Grove, CA).

2.2. cDNA constructs

OA1 cDNA cloned into the EcoRI-NotI site of pCDNA 3.2/V5-DEST (invitrogen, CA), was donated by Dr. Christopher E. Touloukian (Indiana University, Indianapolis, IN). pMS402 and pMS411 are bacterial expression vectors encoding the OA1 gene. pMS420 is a mammalian expression vector carrying the OA1 gene, and pMS430 contains the OA1 gene fused to the N-terminal region of GFP on a mammalian expression vector. For the construction of pMS402, a 1.2-kilobase pair BglII-EcoRI fragment containing OA1 gene was excised from the Touloukian plasmid described above and cloned into the BamHI-EcoRI site of pBluescriptII-SK(−) (Stratagene, La Jolla, CA). The site generated by the ligation between BglII site from OA1 gene fragment and BamHI site from pBluescriptII-SK(−) (GGATCT) was mutated to a BglII site (*A*GATCT). For the construction of pMS420, the 1.2-kilobase pair BglII-EcoRI fragment from pMS411 containing the OA1 gene was cloned into BglII-EcoRI site of pAcGFP-N1 (BD Bioscience). Similarly, pMS421, pMS422, pMS423, pMS424, pMS425, pMS426, pMS427, and pMS428 were constructed by cloning the corresponding BglII-EcoRI fragment from pMS412, pMS413, pMS414, pMS415, pMS416, pMS417, pMS418, and pMS419, respectively, into the BglII-EcoRI site of pAcGFP-N1. pMS412, pMS413, pMS414, pMS415, pMS416, pMS417, pMS418, and pMS419 were constructed as indicated below in "*Site-directed Mutagenesis*". For the construction of pMS430, the stop codon (TGA) at the C-terminus of the OA1 gene on pMS420 was mutated to AGA. As to pMS431, pMS432, pMS433, pMS434, pMS435, pMS436, pMS47, and pMS438, their stop codons (TGA) at the C-terminus of OA1 gene were mutated as well.

pMS445 carries the LAMP1 (lysosome associated membrane protein 1) gene on pBluescriptII-SK(−). For its construction, a 1.5-kilobase pair XhoI-BamHI fragment containing the LAMP1 gene was excised from pSLVhL1 (Williams and Fukuda, 1990), which was provided by Dr. Minoru Fukuda (Cancer