Supplemental Information

Contents

1. Merged PDF file containing:

   A. Supplemental Figure and Legend
   Figure S1. Translocation of Arrestin in Response to Light
   Demonstrates the location of arrestin in photoreceptor cells under the lighting conditions used to isolate PSC complexes.

   B. Supplemental Tables S5-S7
   Table S5. Summary of Immunofluorescence Validation for Novel Putative PSC Complex Proteins
   Table S6. Candidate Disease Genes
   Table S7. Photoreceptor Proteins Used for Benchmarking the Quantitative Analysis

2. Supplemental Data
   Table S1. The complete mouse PSC complex proteome
   Excel spreadsheet; includes the following worksheets:
   A – Complete PSC Complex Proteome
   B – PSC-OS group of proteins
   C – PSC-IS group of proteins
   D – Rootletin KO only proteins
   E – Hypothetical proteins
   F – Notes, with legends for A-E.

   Tables S2-S4: Summaries of LC-MS/MS analyses, including MS/MS data and peptide sequences for:
   Table S2. Wild-type PSC complex
   Table S3. PSC complex-cytoskeleton
   Table S4. Rootletin KO PSC complex

   Table S8. Quantitative Analysis of PSC-OS Proteins

A. Supplemental Figure

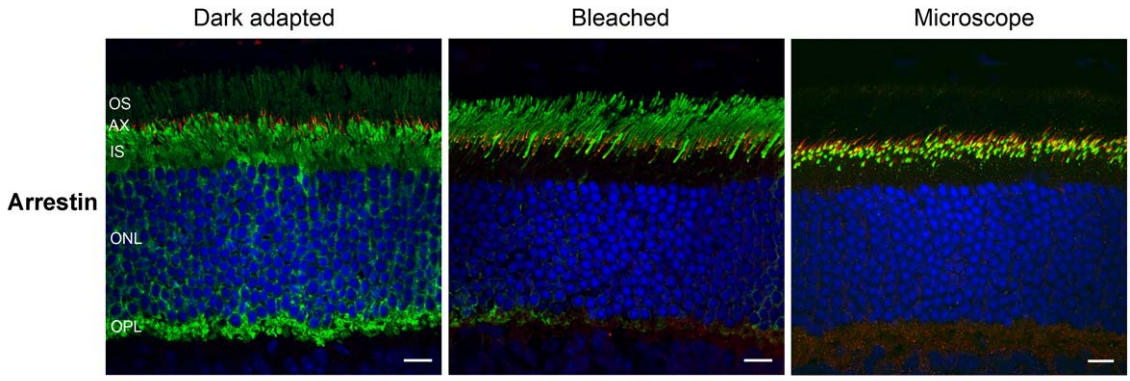

Figure S1. Translocation of Arrestin in Response to Light
Frozen sections of retina were prepared from dark-adapted mice, mice exposed to 15K lux of light for 30 minutes to bleach to photoreceptors, and mice exposed to room light followed by a brief (10 second) exposure to the dissecting microscope light to mimic the conditions used for harvesting PSCs and PSC-cytoskeletons. Sections were stained with antibodies to arrestin (green). All sections were also stained with antibodies to Rp1 in order to visualize the photoreceptor axoneme (red). In the dark adapted state, arrestin in located in the inner segments, cell bodies and synaptic terminals of photoreceptor cells. A strong, bleaching light exposure caused the majority of the arrestin to translocate into the outer segments of rod and cone photoreceptors. A brief exposure to the dissecting microscope light caused partial movement of arrestin, which has moved to the base of the inner segment; little arrestin has reached the OS under these conditions. AX, axoneme; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segment. Bars = 10 μm.

Table S5. Summary of Immunofluorescence Validation for Novel Putative PSC Complex Proteins

OS /IS[a]	Refseq Protein Name	Refseq ID	Total Peptides	Cilia Protein[b]	Antibody	Source	Location[c]	Reference
IS	CLIP associating protein 1	XP_889298.1	21	Shared	2292	Fedor Severin	PSC	*J Cell Biol.* 168:141-53, 2005
IS	CLIP associating protein 2	NP_083909.1	17		401	Fedor Severin	PSC	
IS	coronin, actin binding protein 1B	NP_035908.1	2		4245	James Bear	PSC	*J Biol Chem.* 280:31913-23, 2005
IS	doublecortin and calcium/calmodulin-dependent pro	NP_064362.1	8		1380	Joseph Gleeson	PSC	*J Neurosci.* 20:9152, 2000
IS	exocyst complex component 4	NP_033174.2	12		Sec8	Stressgen	PSC	
IS	huntingtin interacting protein 1 related	NP_659507.2	11		UM374, 1E1	Theodora S. Ross	PSC	*J Biol Chem.* 279:14294, 2004
IS	kinesin family member 2A	NP_032468.1	6	Shared	Kif2a	Duane A. Compton	PSC	*J Cell Biol.* 166(:473, 2004
IS	myosin IB	NP_034993.2	16		2652	Peter G. Gillespie	PSC	*J Neurosci.* 22:2487, 2002
IS	myosin XVIIIa	NP_035716.1	39		434, 435	Zissis C. Chroneos	PSC	*J Biol Chem.* 278:51448, 2003
IS	outer dense fiber of sperm tails 2	NP_038643.1	3	Shared	ODF2	Sigrid Hoyer-Fender	PSC	*J Cell Sci.* 117:4643, 2004
IS	prosaposin	NP_035309.2	7		mSapD, mPS	Grabowski GA	PSC	*Mol Genet Metab.* 76:271, 2002
IS	RAB11a, member RAS oncogene family	NP_059078.2	9	Shared	VU57	Lynne A. Lapierre	PSC	*Exp Cell Res.* 290:322, 2003
IS	synaptopodin 2	XP_001005167.1	7		Synpo2	Peter Mundel	PSC	*J Cell Biol.* 155:393, 2001
IS	syntrophin, basic 1	NP_057876.1	4		SYN258	Marvin Adams	PSC	
IS	tropomyosin 4	NP_001001491.1	5		tropomyosin4	Chemicon	PSC	
IS	microtubule-associated protein 7	NP_032661.1	10		Map7-N, Map7-C	Makoto Suzuki	Muller	*J. Biol. Chem.* 278: 51448, 2003
IS	syntrophin, basic 2	NP_033255.1	5		SYN150	Marvin Adams	Syn	
IS	thioredoxin domain containing 5	NP_663342.3	3		eRp46	Marek Michalak	IR, IS, ONL	
OS	Bardet-Biedl syndrome 1 homolog	NP_001028300.1	26	Shared	bbs1	Nicholas Katsanis	PSC	
OS	Bardet-Biedl syndrome 2 homolog	NP_080392.1	22	Shared	bbs2	Nicholas Katsanis	PSC	
OS	Bardet-Biedl syndrome 4 homolog	NP_780534.1	19	Shared	bbs4	Nicholas Katsanis	PSC	
OS	Bardet-Biedl syndrome 5	NP_082560.1	14	Shared	bbs5	Bradley K Yoder	PSC	
OS	Bardet-Biedl syndrome 7	NP_082086.1	31	Shared	bbs7	Nicholas Katsanis	PSC	
OS	dynactin 1	NP_031861.1	35	Shared	p150, 502, 235	Erika Holzbaur	PSC	
OS	dynactin 2	NP_081427.1	12	Shared	p50, 1097	Erika Holzbaur	PSC	
OS	dynamin 2	NP_001034609.1	18		dynammin II	BD Transduction	PSC	
IS	dystonin	NP_598594.2	50	Shared	10C5	Jonathan C. R. Jones	PSC	*J Comp Neurol.* 487:190, 2005
OS	glutathione S-transferase, theta 1	NP_032211.2	5	Shared	mGstt1	Philip Board	PSC	
OS	glutathione S-transferase, theta 2	NP_034491.1	3		hGstt2	Philip Board	PSC	
OS	glutathione transferase zeta 1	NP_034493.1	8		Gstz	Philip Board	PSC	*Am. J Path.* 165:679, 2004
OS	hypoxia up-regulated 1	NP_067370.2	18		512 (Grp170)	Xiang-Yang Wang	PSC	*J Immunol.* 166: 490, 2001
OS	IQ motif containing GTPase activating protein 1	NP_057930.1	3		Iqgap1	Santa Cruz Biotechnology	PSC	
OS	kinectin 1	NP_032503.1	36		mIn3, mIn2	Hanry Yu	PSC	*J Cell Sci.* 117: 4537, 2004
OS	microtubule-actin crosslinking factor 1	XP_890015.2	135		CU119	Ronald K. H. Liem	PSC	*J Cell Sci.* 118:3727, 2005
OS	myosin, heavy polypeptide 9, non-muscle	NP_071855.1	39	Shared	MHC II A	Covance Research Products	PSC	
OS	myosin, heavy polypeptide 10, non-muscle	NP_780469.1	45	Shared	MHC II B	Covance Research Products	PSC	
OS	N-ethylmaleimide sensitive fusion protein	NP_032766.2	38		Nsf	Masato Hirata	PSC	*Mol Cell Neurosci.* 30:197, 2005
OS	neurofascin	NP_874385.1	17		NFC	Peter J. Brophy	PSC	*J Cell Biol.* 150: 657, 2000
OS	plectin 1	NP_035247.1	78	Shared	#46	Gerhard Wiche	PSC	*J Invest Dermatol.* 120:189, 2003
OS	Rab acceptor 1	NP_034391.1	2		Prenylin (RPA1)	Guangpu Li	PSC	*Biochem. Biophy. Res. Commun.* 275, 509, 2000
OS	RAB11B, member RAS oncogene family	NP_033023.1	12		VU96	Lynne A. Lapierre	PSC	*Exp Cell Res.* 290:322, 2003
OS	restin	NP_062739.2	15	Shared	2360, 1024	Niels Galjart	PSC	
OS	septin 5	NP_998779.1	12		Sept5	Bill Trimble	PSC	
OS	septin 11	NP_001009818.1	16		Sept11	Bill Trimble	PSC	
OS	NS1-associated protein 1	NP_062640.1	15		7772	Michael Mueckler	PSC	*J. Biol. Chem.* 277:25233, 2002
OS	tetratricopeptide repeat domain 8	NP_083829.1	15	Shared	bbs8	Nicholas Katsanis	PSC	
OS	thioredoxin domain containing 12	NP_079610.1	3		eRp19	Marek Michalak	PSC	*Mol. Cell. Proteomics* 2:1104, 2003
OS	transgelin 3	NP_062728.1	8		NP25	Yukio Okano	PSC	*Neurosci Res.* 48:439, 2004
OS	chondroitin sulfate proteoglycan 4	NP_620570.1	11		NGC	Atsuhiko Oohira	IR	*J Biol Chem.* 270:26876, 1995
OS	septin 2	NP_035021.1	8	Shared	Sept2	Bill Trimble	IR, IS	
OS	septin 3	NP_036019.1	10		Sept3	Bill Trimble	IR	

Notes: [a] IS, PSC-IS; OS, PSC-OS

[b] Cilia Protein: Shared with CiliaProteome database

[c] Locations: IR, inner retina; ONL, outer nuclear layer; PSC, specific PSC complex signal on frozen section and or isolated PSC complexes; SYN, synapse

Table S6. Candidate Disease Genes

OMIMID	DISORDER	Mouse Gene	Human Gene	Human Symbol	Protein Name	Human Map Location	Source	Reference
Retinal Disorders								
215500	Central areolar choroidal dystrophy	13681	1973	EIF4A1	eukaryotic translation initiation factor 4A, isoform 1	17p13	PSC-IS	*Cytogenet Cell Genet.* 93:43, 2001
605750	Exudative vitreoretinopathy-3	20511	6506	SLC1A2	solute carrier family 1, member 2	11p13-p12	PSC-OS	*Am. J. Hum. Genet.* 68: 778, 2001
604537	Leber congenital amaurosis 5	75782	167691	C6ORF152	hypothetical protein LOC167691	6q11-q16	PSC-OS	*Am. J. Hum. Genet.* 66: 319, 2000
		13627	1915	EEF1A1	eukaryotic translation elongation factor 1 alpha 1	6q11-q16	PSC-OS	
		382090	22832	KIAA1009	KIAA1009 protein	6q11-q16	PSC-IS	
		17920	4646	MYO6	myosin VI	6q11-q16	PSC-OS	
		116837	22999	RIMS1	regulating synaptic membrane exocytosis 1	6q11-q16	PSC-OS	
		212531	83699	SH3BGRL2	SH3 domain binding glutamic acid-rich protein like 2	6q11-q16	PSC-IS	
		20616	9892	SNAP91	synaptosomal-associated protein 91	6q11-q16	PSC-OS	
		56403	10492	SYNCRIP	NS1-associated protein 1	6q11-q16	PSC-OS	
		69981	55754	TMEM30A	transmembrane protein 30A	6q11-q16	PSC-OS	
153840	Macular dystrophy, atypical vitelliform	239528	27161	EIF2C2	eukaryotic translation initiation factor 2C, 2	8q24	PSC-IS	*Am. J. Hum. Genet.* 35: 78, 1983
		18810	5339	PLEC1	plectin 1	8q24	PSC-OS	
300424	Retinitis pigmentosa 23	57394	57393	TMEM27	transmembrane protein 27	Xp22	PSC-OS	*Invest. Ophthalmol. Vis. Sci.* 41:2080, 2000
300605	Retinitis pigmentosa 34	320707	492	ATP2B3	ATPase, Ca++ transporting, plasma membrane 3	Xq28	PSC-IS	*J. Med. Genet.* 43: e27, 2006
		27061	10134	BCAP31	B-cell receptor-associated protein 31	Xq28	PSC-OS	
		192176	2316	FLNA	filamin A, alpha (actin binding protein 280)	Xq28	PSC-OS	
		67391	65991	FUNDC2	FUN14 domain containing 2	Xq28	PSC-OS	
		14567	2664	GDI1	guanosine diphosphate (GDP) dissociation inhibitor 1	Xq28	PSC-OS	
		14539	2652	OPN1MW	opsin 1 (cone pigments), medium-wave-sensitive	Xp28	PSC-OS	
		67790	116442	RAB39B	RAB39B, member RAS oncogene family	Xq28	PSC-OS	
		20832	6748	SSR4	signal sequence receptor, delta	Xq28	PSC-OS	
		22327	7411	VBP1	von Hippel-Lindau binding protein 1	Xq28	PSC-OS	
192315	Retinopathy, vascular; cerebral/renal involvement	97541	5859	QARS	glutaminyl-tRNA synthetase	3p21.3-p21.1	PSC-IS	*Am. J. Hum. Genet.* 69:447, 2001
193235	Vitreoretinopathy, neovascular inflammatory	13043	2017	CTTN	cortactin	11q13	PSC-OS	*Hum. Molec. Genet*. 1: 685, 1992
Other Eye Disorders								
310460	Bornholm eye disease/Myopia-1	320707	492	ATP2B3	ATPase, Ca++ transporting, plasma membrane 3	Xq28	PSC-IS	*Clin. Genet.* 38: 281, 1990
		27061	10134	BCAP31	B-cell receptor-associated protein 31	Xq28	PSC-OS	
		26370	1069	CETN2	centrin, EF-hand protein, 2	Xq28	PSC-OS	
		192176	2316	FLNA	filamin A, alpha (actin binding protein 280)	Xq28	PSC-OS	
		67391	65991	FUNDC2	FUN14 domain containing 2	Xq28	PSC-OS	
		14567	2664	GDI1	guanosine diphosphate (GDP) dissociation inhibitor 1	Xq28	PSC-OS	
		53332	8776	MTMR1	myotubularin related protein 1	Xq28	PSC-IS	
		67790	116442	RAB39B	RAB39B, member RAS oncogene family	Xq28	PSC-OS	
		20832	6748	SSR4	signal sequence receptor, delta	Xq28	PSC-OS	
		22327	7411	VBP1	von Hippel-Lindau binding protein 1	Xq28	PSC-OS	
156850	Cataract, congenital, with microphthalmia	268932	57524	CASKIN1	CASK interacting protein 1	16p13.3	PSC-OS	*Hum. Genet.* 90: 177, 1992.
		15369	3163	HMOX2	heme oxygenase (decycling) 2	16p13.3	PSC-IS	
		106633	9742	IFT140	intraflagellar transport 140 homolog	16p13.3	PSC-OS	
		30957	23162	MAPK8IP3	mitogen-activated protein kinase 8 interacting protein 3	16p13.3	PSC-IS	
		214952	89941	RHOT2	ras homolog gene family, member T2	16p13.3	PSC-OS	
		66049	79641	ROGDI	rogdi homolog (Drosophila)	16p13.3	PSC-IS	
		68015	10131	TRAP1	TNF receptor-associated protein 1	16p13.3	PSC-OS	
217400	Corneal dystrophy and perceptive deafness 1	77006	65992	C20orf116	chromosome 20 open reading frame 116	20p13	PSC-OS	*J. Med. Genet.* 39: 110, 2002.
		67134	10528	NOL5A	nucleolar protein 5A	20p13	PSC-IS	
		386649	55968	NSFL1C	NSFL1 (p97) cofactor (p47)	20p13	PSC-IS	
		19261	140885	SIRPA	signal-regulatory protein alpha	20p13	PSC-IS	
602082	Corneal dystrophy, Thiel-Behnke type	107338	8729	GBF1	golgi-specific brefeldin A resistance factor 1	10q24	PSC-IS	*Genomics* 46: 152, 1997.
		73728	5662	PSD	pleckstrin and Sec7 domain containing	10q24	PSC-IS	
126800	Duane syndrome	211673	10565	ARFGEF1	ADP-ribosylation factor guanine nucleotide-exchange factor 1	8q13	PSC-IS	*Hum. Genet.* 108: 398, 2001
		70675	80124	VCPIP1	valosin containing protein (p97)/p47 complex interacting protein 1	8q13	PSC-IS	
164100	Nystagmus-2, autosomal dominant	15516	3326	HSP90AB1	heat shock protein 90kDa alpha, class B member 1	6p12	PSC-OS	*Am. J. Ophthal.* 125: 64, 1998.
Renal Disorders								
161900	Nephropathy-hypertension	213498	9826	ARHGEF11	Rho guanine nucleotide exchange factor (GEF) 11	1q21	PSC-IS	*Am. J. Hum. Genet.* 67: 647, 2000.
		23831	23632	CA14	carbonic anhydrase XIV	1q21	PSC-OS	
		17827	4580	MTX1	metaxin 1	1q21	PSC-IS	
		229589	58497	PRUNE	prune homolog (Drosophila)	1q21	PSC-OS	
Deafness								
120502	Branchiootic syndrome 2 (BOS2)	14645	2752	GLUL	glutamate-ammonia ligase (glutamine synthetase)	1q31	PSC-OS	*Am. J. Hum. Genet.* 66: 1715, 2000.
608372	Deafness, autosomal dominant 49 (DFNA49)	98660	477	ATP1A2	ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide	1q21-q23	PSC-OS	*J. Med. Genet.* 40: 832, 2003.
607239	Deafness, autosomal recessive 33 (DFNB33)	227634	157922	CAMSAP1	calmodulin regulated spectrin-associated protein 1	9q34.3	PSC-IS	*Europ. J. Hum. Genet.* 10: 391, 2002.
		227648	9919	KIAA0310	KIAA0310	9q34.3	PSC-IS	
605429	Deafness, nonsyndromic, modifier 1 (DFNM1)	11931	481	ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide	1q24	PSC-OS	*Nature Genet.* 26: 431, 2000.
		171567	29922	NME7	non-metastatic cells 7, protein expressed in	1q24	PSC-OS	
304500	Deafness, X-linked 2, perceptive congenital (DFN2)	18823	5354	PLP1	proteolipid protein (myelin) 1	Xq22	PSC-IS	*Hum. Molec. Genet.* 5: 2055, 1996
300066	Deafness, X-linked 6, sensorineural (DFN6)	57394	57393	TMEM27	transmembrane protein 27	Xp22	PSC-OS	*Hum. Molec. Genet.* 5: 1383, 1996
Neurodegenerative Disorders								
108600	Spastic ataxia, autosomal dominant (SAX1)	12034	11331	PHB2	prohibitin 2	12p13	PSC-OS	*Am. J. Med. Genet.* 131A: 249, 2004.
600223	Spinocerebellar ataxia-4 (SCA4)	102339	25839	COG4	component of oligomeric golgi complex 4	16q22.1	PSC-IS	*J. Neurol.* 250: 668, 2003
		13680	55308	DDX19A	DEAD (Asp-Glu-Ala-As) box polypeptide 19A	16q22.1	PSC-OS	
		234663	1783	DYNC1LI2	dynein, cytoplasmic 1, light intermediate chain 2	16q22.1	PSC-OS	
		234699	23644	EDC4	enhancer of mRNA decapping 4	16q22.1	PSC-OS	
		116733	27183	VPS4A	vacuolar protein sorting 4A	16q22.1	PSC-IS	

Table S7. Photoreceptor Proteins Used for Benchmarking the Quantitative Analysis

Protein	Spectra PSC[a]	Predicted Peptides[b]	S/PP	Rho/Protein[c]	Adjusted Rho/Protein[d]	Protein/Rho[e]	Molecules/ Rod[f]	Notes	Reference
Rho	494	1	494.00	1.00	1.00	1.00E+00	7.00E+07	(1)	*Vision Res.* 44:3235, 2004
Gnat1	2097	16							
Gnb1	602	15							
Gngt1	19	4							
Transducin Holoprotein	2718	35	77.66	12.00	14.00	7.14E-02	5.00E+06	(2)	*Science* 282:117, 1998; Neuron 34:95, 2002
Sag	1276	20	63.80	1.28	25.60	3.91E-02	2.73E+06	(3)	*J. Neurosci.* 26:1146, 2006
Pde6a	274	53							
Pde6b	568	48							
Pde6g	7	2							
PDE Holoprotein	849	103	8.24	65.00	65.00	1.54E-02	1.08E+06	(4)	*J. Neurosci.* 23:1287, 2003
Rds	136	13	10.46	180.00	180.00	5.56E-03	3.89E+05		*Biochemistry* 35:6144, 1996
Rom1	78	8	9.75	180.00	180.00	5.56E-03	3.89E+05		*Biochemistry* 35:6144, 1996
Grk1	218	25	8.72	600.00	600.00	1.67E-03	1.17E+05		*J. Biol. Chem.* 270:16147, 1995;
Pdc	72	13	5.54	14.00	120.00	8.33E-03	5.83E+05	(5)	*J. Biol. Chem.* 279:19149, 2004
Gucy2e	229	46	4.98	260.00	260.00	3.85E-03	2.69E+05		*Eur. J. Biochem.* 270:3814, 2003
Abca4	402	104	3.87	120.00	120.00	8.33E-03	5.83E+05		*Nat. Genet.* 17:15, 1997
Rcvrn	73	14	5.21	12.50	104.00	9.62E-03	6.73E+05	(6)	*J. Biol. Chem.* 280:29250, 2005
Slc24a1	202	44	4.59	800.00	800.00	1.25E-03	8.75E+04		*J. Biol. Chem.* 263:11382, 1998
Rgs9	97	42	2.31	610.00	610.00	1.64E-03	1.15E+05		*J. Neurosci.* 23:1287, 2003
Guca1b	16	8	2.00	1100.00	1100.00	9.09E-04	6.36E+04		*Eur. J. Biochem.* 270:3814, 2003
Guca1a	22	12	1.83	900.00	900.00	1.11E-03	7.78E+04		*Eur. J. Biochem.* 270:3814, 2003

[a] **Spectra PSC**	Total number of MS/MS spectra observed from all specific peptides from designated protein.
[b] **Predicted Peptides**	Determined as described in Supplementary Methods
[c] **Rho/Protein**	Reported amount of protein from literature, expressed in terms of ratio to rhodopsin
[d] **Adjusted Rho/Protein**	Value from Rho/Protein column adjusted for individual proteins; see Notes below
[e] **Protein/Rho**	Inverse of Adjusted Rho/Protein value
[f] **Molecules/Rod**	Protein/Rho multiplied by 7E+07
Notes	
1	The spectral count data for rhodopsin used for this analysis are derived from the more hydrophilic C-terminus of the protein, which generates a single tryptic peptide (NPLGDDDASATASK)
2	Immunofluorescence analysis (not shown) showed 85% transducin in outer segment under lighting conditions used for PSC complex isolation.
3	Immunofluorescence analysis (Figure S1) showed 5% of arrestin in outer segment under lighting conditions used for PSC complex isolation.
4	Stoichiometry of subunits 1:1:2
5	12.5% of phosducin is reported to be located in the outer segment
6	Since transducin and arrestin were found to be in their dark-adapted locations under the lighting conditions used for PSC complex isolation, it was assumed that recoverin was also in its dark-adapted location, with 12% of the total protein in the outer segment