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Centrins in retinal photoreceptor cells: Regulators in the connecting cilium

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Abstract

Changes in the intracellular Ca^{2+} concentration regulate the visual signal transduction cascade directly or more often indirectly through Ca^{2+}-binding proteins. Here we focus on centrins, which are members of a highly conserved subgroup of the EF-hand superfamily of Ca^{2+}-binding proteins in photoreceptor cells of the vertebrate retina. Centrins are commonly associated with centrosome-related structures. In mammalian retinal photoreceptor cells, four centrin isoforms are expressed as prominent components in the connecting cilium linking the light-sensitive outer segment compartment with the metabolically active inner segment compartment. Our data indicate that Ca^{2+}-activated centrin isoforms assemble into protein complexes with the visual heterotrimeric G-protein transducin. This interaction of centrins with transducin is mediated by binding to the $\beta\gamma$-dimer of the heterotrimeric G-protein. More recent findings show that these interactions of centrins with transducin are reciprocally regulated via site-specific phosphorylations mediated by the protein kinase CK2. The assembly of centrin/G-protein complexes is a novel aspect of translocation regulation of signalling proteins in sensory cells, and represents a potential link between molecular trafficking and signal transduction in general.

Keywords: Ca^{2+}-binding proteins; Centrins; Retina; Photoreceptor cells; Transducin; Light-dependent translocation

Contents

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1. Introduction

Cone and rod photoreceptor cells are highly polarized specialized neurons, which consist of morphologically and functionally distinct cellular compartments (Fig. 1). The light-sensitive photoreceptor outer segment is linked with an inner segment via a modified, non-motile cilium, termed connecting cilium (Fig. 1). The inner segment contains the organelles typical for the metabolism of eukaryotic cells and continues into the perikaryon and the synaptic region. The outer segment contains all components of the visual transduction cascade and is arranged as hundreds of stacked membrane disks. Signal transduction in vertebrate rod cells starts with the light-induced formation of active rhodopsin (Rh*), which interacts with the visual G-protein transducin (G_t). G_t activation by Rh* represents a key amplification step in the cascade, in which a single Rh* can catalyze the activation of hundreds of G_t molecules (Fung and Stryer, 1980; Heck and Hofmann, 2001). Thereby, Rh* interacts with the GDP-bound form of the intact G_t holoprotein ($G_t\alpha$-GDP-$G_t\beta\gamma$ or $G_t$holo), and initiates the light-dependent transduction process by triggering the rapid exchange of bound GDP for GTP on the α-subunit ($G_t\alpha$). This is followed very rapidly by the dissociation of the $G_t\alpha$-GTP (or G*) from the Rh* as well as from the membrane bound $\beta\gamma$-heterodimeric subunit ($G_t\beta\gamma$). Activated $G_t\alpha$-GTP stimulates the activity of its effector enzyme, the cGMP phosphodiesterase (PDE), also known as PDE6 (Beavo, 1995), which in turn hydrolyzes cGMP to 5′-GMP, leading to the closure of cGMP-gated channels (CNG channels) localized in the plasma membrane (Heck and Hofmann, 1993; Okada et al., 2002). The closure of CNG channels leads to a drop of the cationic current (carried by Na^+ and Ca^{2+}) resulting in the hyperpolarization of the cell membrane and the decrease of transmitter release from the synaptic terminal (Molday and Kaupp, 2000). The recovery phase of the visual transduction cascade and light adaptation of photoreceptor cells rely on changes in the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$. It is well established that changes in $[Ca^{2+}]_i$ affect portions of the phototransduction cascade indirectly through Ca^{2+}-binding proteins (Palczewski et al., 2000).

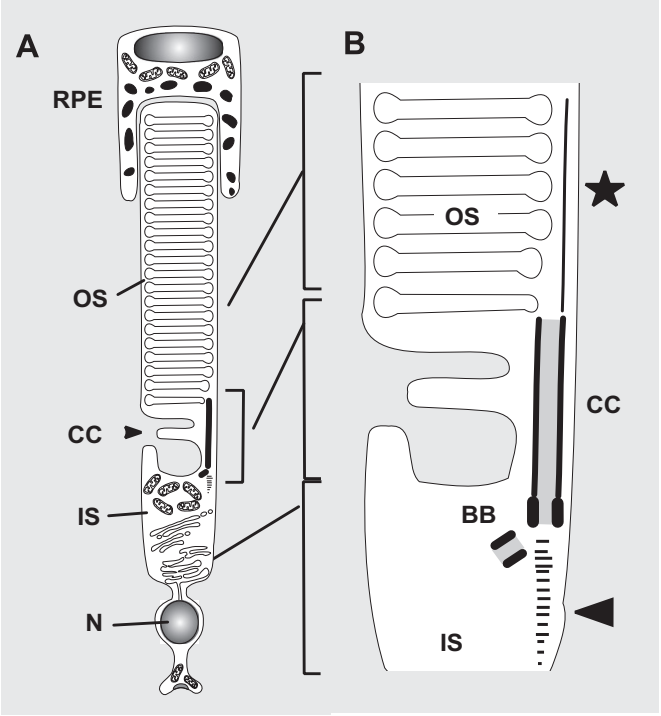

Fig. 1. Schematic representation of a vertebrate rod photoreceptor cell. (A) The photoreceptor cell is composed of a light-sensitive outer segment (OS) which is linked via a specialized non-motile cilium, the connecting cilium (CC) with the inner segment (IS). The OS tips are attached to the cells of the retinal pigment epithelium (RPE). The IS contains all organelles necessary for biosynthesis of the cell. (B) Schematic representation of the ciliary apparatus of a photoreceptor cell. The ciliary apparatus is composed of the CC, which represents an elongated transition zone, and the basal body complex (BB). Ciliary axonemal microtubules (*asterisk*) project from the CC into the OS. The ciliary rootlet (*arrowhead*) project from the basal body complex into the IS of the photoreceptor cell. Centrin localizations in the CC and at the BB are indicated in grey.

The membranous outer segment disks which contain the signal transduction machinery are continually renewed throughout lifetime (Young, 1976). Newly synthesized disks are added at the base of the outer segment (Steinberg et al., 1980; Usukura and Obata, 1996) whereas disks at the distal tip of the outer segment are phagocytosed by cells of the retinal pigment epithelium (Young, 1976). This permanent turnover requires effective mechanisms for the transport of newly synthesized components from cell organelles of biosynthesis in the inner segment, to the outer segment, the cell compartment of signal transduction (Sung and Tai, 2000). After delivery, some molecules of the outer segments, e.g. membrane proteins (ion transporters, channels, as well as the visual pigment rhodopsin) stay there permanently, whereas other molecules of the signal transduction cascade, e.g. arrestin and transducin, exhibit massive light-dependent reversible translocations between

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photoreceptor compartments (Brann and Cohen, 1987; Philp et al., 1987; Whelan and McGinnis, 1988; Organisciak et al., 1991; McGinnis et al., 2002; Pulvermüller et al., 2002; Sokolov et al., 2002, 2004; Wolfrum et al., 2002; Mendez et al., 2003; Peterson et al., 2003). These bidirectional translocations of components of the transduction cascade are thought to contribute to slow but long lasting adaptation of photoreceptor cells (Sokolov et al., 2002, 2004; Hardie, 2003; Frechter and Minke, 2006).

All intracellular exchanges between these two functional compartments of vertebrate photoreceptor cells occur through the slender connecting cilium, which is the only cytoplasmic bridge between the inner segment and the outer segment (Fig. 1). During recent years, an increasing number of proteins and protein complexes have been identified at the ciliary apparatus of vertebrate photoreceptor cells, which is composed of the connecting cilium and the basal body complex (Liu et al., 2007; Roepman and Wolfrum, 2007). The identified molecules of the cilium were very often suggested to play a role in the ciliary transport (Schmitt and Wolfrum, 2001; Stohr et al., 2003; Liu et al., 2007; Roepman and Wolfrum, 2007). The list of molecules identified in the photoreceptor cilium contains proteins from different classes and families, but also includes molecular motors associated with microtubules and actin filaments and represent good candidates for the active molecular transport through the connecting cilium (e.g. myosin VIIa and kinesin II) (Liu et al., 1997, 1999; Marszalek et al., 2000; Wolfrum and Schmitt, 2000; Williams, 2002; Luby-Phelps et al., 2007). In addition, all four known centrin isoforms are also found in the ciliary apparatus of rodent photoreceptor cells (Gießl et al., 2004a, b, 2006).

The present review deals with the current view of structure, expression, subcellular localization and function of centrins. In particular, we focus on these aspects of the small Ca^{2+}-binding centrins in the vertebrate retina. The prominent localization of centrin isoforms is described in the connecting cilium of the photoreceptor cell and the putative role of centrin/transducin protein complexes in the regulation of transducin movements through the cilium is discussed.

2. Centrin genes and protein structures

2.1. Centrin genes and proteins in their phylogenic context

Centrins, also termed "caltractins", are highly conserved low molecular weight phospho-proteins (Salisbury, 1995; Schiebel and Bornens, 1995; Gießl et al., 2004a). They belong to the large EF-hand superfamily of Ca^{2+}-binding proteins which includes parvalbumin, troponin C, the S100 protein and the well-known Ca^{2+}-sensor calmodulin (Kretsinger and Nockolds, 1973; Kretsinger, 1976; Persechini et al., 1989; Nakayama and Kretsinger, 1994). Centrins were first described in unicellular green algae, where they are associated with the basal apparatus of flagella (Salisbury et al., 1984). In these protists, centrins are thought to participate in Ca^{2+}-dependent contractions of striated flagellar rootlets (Salisbury et al., 1984). More recently, centrins have been found to be ubiquitously associated with centrioles of basal bodies and centrosomes, as well as mitotic spindle poles in cells from diverse eukaryotic organisms, from yeast to man (Salisbury, 1995; Schiebel and Bornens, 1995). The centrin protein family is one class of about 350 "eukaryotic signature proteins" (ESPs) that occur in all eukaryotic cells but have no significant homology to proteins in archaea and bacteria (Hartman and Fedorov, 2002; Salisbury, 2007). These ESPs define an ancient class of proteins that might be uniquely critical for the structure and function of the eukaryotic cell in general (Hartman and Fedorov, 2002).

Over the last two decades, centrin genes were described in a large variety of species from all kingdoms of eukaryotic organisms, protists, fungi, plants and animals (Huang et al., 1988a; Baum et al., 1986, 1988; Lee and Huang, 1993; Errabolu et al., 1994; Zhu et al., 1995; Levy et al., 1996; Madeddu et al., 1996; Meng et al., 1996; Middendorp et al., 1997; Wottrich, 1998; Daunderer et al., 2001; Gavet et al., 2003; Guerra et al., 2003; Correa et al., 2004; Lemullois et al., 2004; Ribichich and Gomes, 2005; Nagamune and Sibley, 2006; Boutet et al., 2008). Comparisons of amino acid sequences deduced from cDNA clones certainly show that centrins are highly conserved, and yet distinct members of a subfamily of the EF-hand superfamily of Ca^{2+}-binding proteins, also termed the parvalbumin or troponin C superfamily (Fig. 2) (Kretsinger and Nockolds, 1973; Kretsinger, 1976; Persechini et al., 1989; Nakayama and Kretsinger, 1994). Centrins are small acidic proteins (~170 amino acids in length; apparent molecular mass ~20 kDa) (Salisbury, 1995; Schiebel and Bornens, 1995).

To date, in lower eukaryotes like yeast, only one centrin gene (e.g. *Saccharomyces cerevisiae*: *ScCDC31*) has been identified (Baum et al., 1986, 1988). In the unicellular algae *Chlamydomonas reinhardtii*, database searches also provide one centrin gene listed as *CrCEN* or *VFL2*. However, in the proteomic analysis of isolated *Chlamydomonas* centrioles, Keller et al. (2005) identified two other centrins related to mammalian Cen2p and 3p in addition to the previously found centrin (CrCenp/Vfl2p) (Keller et al., 2005). Whereas for lower vertebrate species, an incomplete (most probably) set of one to two centrin genes are deposited in databases, in mammals up to four centrin genes have been described (Fig. 2A) (Friedberg, 2006). In the rodents *Mus musculus* and *Rattus norvegicus* four centrin genes (*MmCetn1–4*; *RnCetn1–4*) were identified (Lee and Huang, 1993; Middendorp et al., 1997; Gavet et al., 2003; Trojan, 2003). In the human genome, three centrin genes are present (*HsCETN1–3*). A predicted fourth centrin gene is found on chromosome 4 (accession number: XR_015512), but the potential gene transcript encodes a very short, 98 amino acids long peptide (Gießl, 2004). It is doubtful whether this transcript will exist as a functional polypeptide in the cell (Gießl, 2004).

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Cluster analyses of the deduced amino acid sequences of the diverse centrins of different organisms reveal several phylogenetic groups within the centrin protein family (Fig. 2). While some centrins of protists cannot be classified to homogeneous groups, most centrins of higher plants, green algae and all vertebrate centrin isoforms form phylogenetic groups. In mammals, Cen1p, 2p and 4p isoforms are very closely related exhibiting high amino acid identities (Table 1). In contrast, sequences of vertebrate Cen3p isoforms, related to the yeast centrin (ScCdc31p), only have high amino acid identities among each other (Table 1, Fig. 2). In mammals, Cen1p, 2p and 4p isoforms are closer related to algal centrin (e.g. *Chlamydomonas* CrCenp/Vfl2p) than to the mammalian Cen3p isoform. This strongly suggests two divergent centrin subfamilies (Middendorp et al., 1997): one centrin subfamily grouped around the mammalian Cen1p, 2p and 4p and another centrin subfamily related to yeast centrin Cdc31p. Since the separation of both centrin subfamilies is already implemented in the unicellular green algae (see above; Keller et al. 2005), this division was a very early event in the molecular evolution of eukaryotes.

2.2. Primary domain structure of centrins

Analyses of the primary structures of centrins demonstrate that the most characteristic domains are the four helix–loop–helix EF-hand consensus motifs (Fig. 2C). These potential Ca^{2+}-binding sites define centrins as members of the superfamily of EF-hand Ca^{2+}-binding

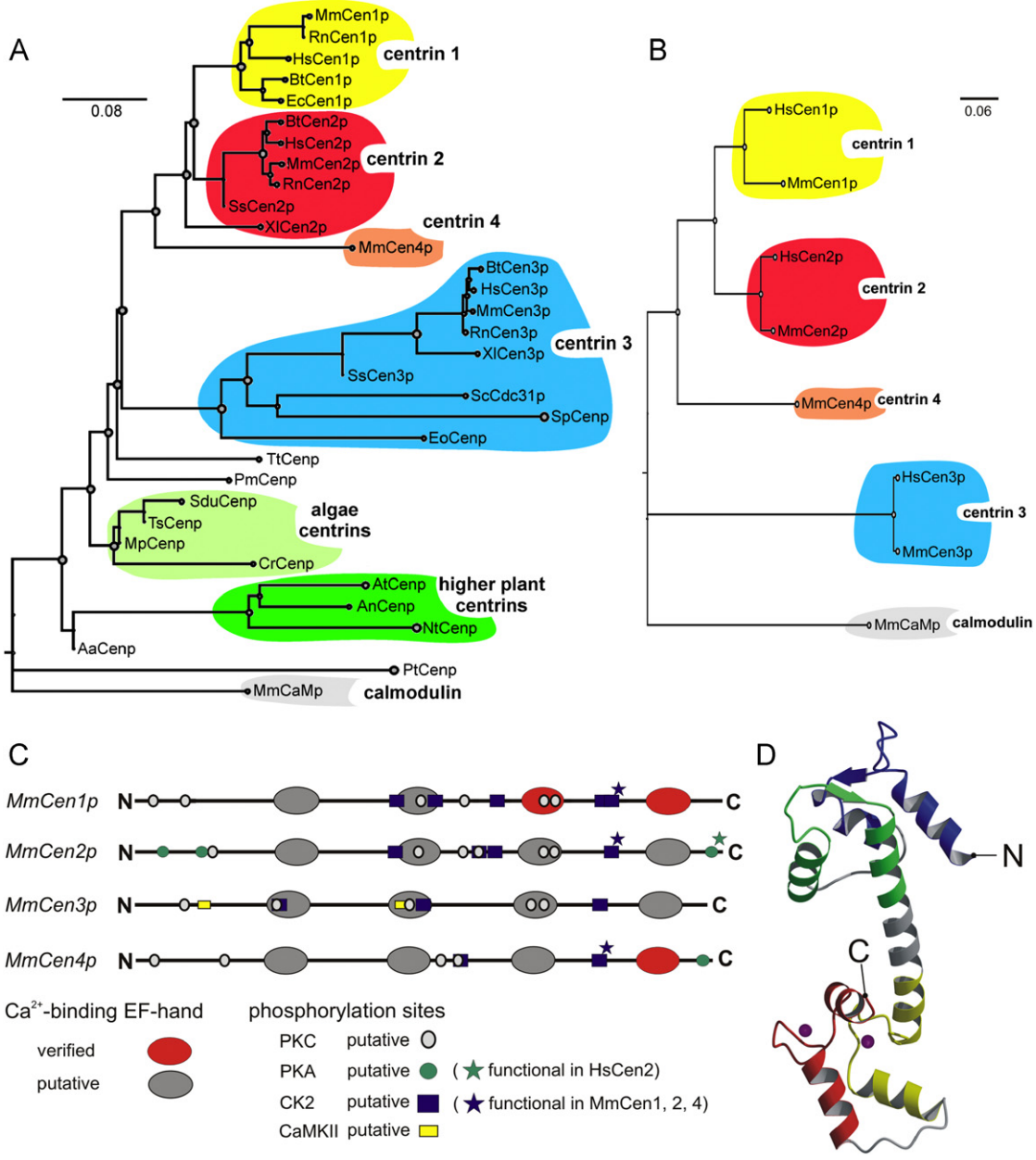

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Table 1
Identity of human and murine centrin isoforms

	CAA43674 MmCaMp	NP_004057 HsCen1p	P41208 HsCen2p	NP_004356 HsCen3p	NP_031619 MmCen1p	NP_062278 MmCen2p	NP_031710 MmCen3p	NP_665824 MmCen4p	Protein
MmCalm1 X61432		52	50	40	51	50	40	46	MmCaMp genbank:CAA43674
HsCETN1 NM_004066	*58*		83	53	90	84	53	69	HsCen1p genbank:NP_004057
HsCETN2 BC013873	*57*	*76*		52	81	95	52	69	HsCen2p genbank:P41208
HsCETN3 NM_004365	*51*	*59*	*60*		52	52	98	49	HsCen3p genbank:NP_004356
MmCetn1 NM_007593	*57*	*84*	*77*	*58*		81	52	67	MmCen1p genbank:NP_031619
MmCetn2 NM_019405	*57*	*76*	*90*	*60*	*78*		52	70	MmCen2p genbank:NP_062278
MmCetn3 NM_007684	*49*	*59*	*60*	*89*	*59*	*59*		49	MmCen3p genbank:NP_031710
MmCetn4 NM_145825	*56*	*66*	*68*	*58*	*67*	*69*	*58*		MmCen4p genbank:NP_665824
Coding sequence	*MmCalm1 X61432*	*HsCETN1 NM_004066*	*HsCETN2 BC013873*	*HsCETN3 NM_004365*	*MmCetn1 NM_007593*	*MmCetn2 NM_019405*	*MmCetn3 NM_007684*	*MmCetn4 NM_145825*	

Summary of the percentage identities of human (HsCen1p–3p) and murine (MmCen1p–4p) centrin proteins (non-italicized). In addition murine calmodulin (MmCaMp) was analyzed. The percentage identity of the coding sequences of the human and murine centrin genes (*HsCETN1–3* and *MmCetn1–4*) are summarized in italics. In addition the coding sequence of the murine calmodulin gene was analyzed (*MmCalm1*). For all analyzed proteins (non-italicized) and coding nucleotide sequences (italicized) the accession numbers are indicated. All sequences were analyzed using the Omiga2.0 software (Oxford molecular Ltd.).

proteins (Kretsinger, 1976; Moncrief et al., 1990; Nakayama et al., 1992). Beside the EF-hand motifs, the primary structures of centrins exhibit several putative phosphorylation sites for protein kinases A, C, CK2 and CaMKII (Fig. 2C). Very recent work identified site- and isoform-specific phosphorylation of murine centrins by CK2 (Trojan et al., 2008). Comparison of the centrin sequences reveals only small differences between the isoforms

Fig. 2. Phylogenetic relationship of centrins and molecular structure of centrins. (A) Phylogenetic relationship of centrins from diverse organisms. Comparison of 33 different amino acid sequences of centrins and murine calmodulin. The genetic consensus tree shows the highest frequency of each node of 1000 repetitions. Geneious 3.0 pro software (Biomatters Ltd) divides the centrins into subgroups: vertebrate centrin isoforms 1–4, algae centrins and higher plant centrins and an out grouped subgroup of calmodulin (MmCaMp = *Mus musculus* calmodulin AN: CAA43674; XlCenp2p, 3p = *Xenopus laevis* centrin 2, 3 AN: BC054948, AAG30507; PtCenp = *Paramecium tetraurelia* centrin AN: AAB188752; BtCen1p–3p = *Bos taurus* centrins 1–3 AN: NP001072974, NP001033604, AAI20178; SsCen2p, 3p = *Sus scrofa* centrins 2, 3 AN: AAY33861, AAY67906; EcCen1p = *Elaphodus cephalophus* centrin 1 AN: ABP57024; HsCen1p–3p = *Homo sapiens* centrins 1–3 AN: see (C); MmCen1p–4p = *Mus musculus* centrins 1–4 AN: see (C); RnCen1p–3p = *Rattus norvegicus* centrins (completed with own data) AN: AAK20385, AAK20386, AAK83217; AtCenp = *Arabidopsis thaliana* centrin AN: CAA08773, AnCenp = *Atriplex nummularia* centrin AN: P41210; NtCenp = *Nicotiana tabacum* centrin AN: AAF07221; CrCenp = *Chlamydomonas reinhardtii* centrin (Vfl2p) AN: EDO98562; SduCenp = *Scherffelia dubia* centrin AN: Q06827; MpCenp = *Micromonas pusilla* centrin AN: CAA58718; EoCenp = *Euplotes octocarinatus* centrin AN: CAB40791; TsCenp = *Tetraselmis striata* centrin AN: P43646; ScCdc31p = *Saccharomyces cerevisiae* AN: CAA52609; SpCenp = *Schizosaccharomyces pombe* centrin AN: CAA20670; PmCenp = *Prorocentrum minimum* centrin AN: ABI14404; AaCenp = *Acetabularia acetabulum* centrin AN: AAM00015; TtCenp = *Tetrahymena thermophila* AN: AAF66602). The tree is not complete (AN: accession number). (B) Phylogenetic tree of four mouse and three human centrins. Genious 3.0 pro separates mammalian centrins into the subgroups 1–4, thereby the Cen1p, 2p and 4p cluster closer to each other (tree isolated to mouse calmodulin). (C) Schematic representation of functional domains in murine centrins. Murine centrin isoforms 1–4 (MmCen1p–4p) possess four EF-hand motifs (*ovals*). Functional EF-hands are indicated by *red ovals*. In MmCen1p EF-hands III and IV bind Ca^{2+}, whereas MmCen4p possesses only one functional EF-hand (EF-hand IV). Unfortunately, no data are available for MmCen2p and 3p. All centrins contain a variety of putative phosphorylation sites for protein kinases A, C, CK2 and CaMKII. In MmCen1p six putative PKC phosphorylation sites are predicted (*grey small ovals*). Only one of five putative CK2 phosphorylation sites (*blue squares*) is functional (*blue star*). MmCen2p contains three putative PKA phosphorylation sites (*green ovals*). One of these sites is functional in HsCen2p (*green star*). For MmCen2p no experimental data are available. Six putative PKC sites are present as well (*grey small ovals*). Like in MmCen1p one CK2 phosphorylation site is functional (*blue star*) and three are inactive (*blue squares*). In contrast, MmCen3p contains no functional CK2 site but three inactive ones (*blue squares*). In addition, there are five putative PKC sites (*grey small ovals*) and two putative CaMKII phosphorylation sites (*yellow rectangles*). MmCen4p contains one active CK2 site (*blue star*) and one is inactive. In addition, four putative PKC sites are present. (D) Crystal structure of MmCen1p. The structure of MmCen1p-L (Park et al., 2006, in prep.), shown as ribbon representation. Large parts of the N-terminal sequence and a small portion at the C-terminus are not visible in the electron density map. The structural model shown here comprises residues Asp28 (indicated by 'N') to Lys167 (indicated by 'C', numbering refers to wild-type MmCen1p). EF-hand motifs I–IV are coloured blue, green, yellow and red, respectively. The N-terminal half of the molecule adopts a typical 'closed' conformation, whereas the C-terminal half is in an 'open' conformation. Ca^{2+} ions (*spheres*) are bound to the EF-hands III and IV; EF-hand I is only partially occupied by Ca^{2+}.

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(Table 1) (Gießl et al., 2004a; Salisbury, 2007). In addition to the EF-hand motifs, the most conserved region is the C-terminal half of the centrins, in particular the short C-terminal sequence (-KKTSLY). This sequence could be responsible for general features of centrins, like the positioning at centrosomal structures (e.g. centrosomes, basal bodies or transition zones of cilia). In contrast to the conserved C-terminal domain, the N-terminus, especially the first 20 amino acids, represent the most variable region of the centrin sequences (Hart et al., 1999, 2001; Salisbury, 2007). Therefore, this region has been suggested to be responsible for some functional diversity among centrin species (Bhattacharya et al., 1993; Salisbury, 1995; Wiech et al., 1996; Wolfrum et al., 2002; Gießl et al., 2004b; Yang et al., 2006b).

2.2.1. Ca^{2+}-binding at EF-hand motifs of centrins

The most prominent characteristic of the centrin protein family are the four EF-hand motifs which posses the potential capacity for Ca^{2+}-binding (Fig. 2C). Therefore, it is not surprising that the function of centrins is accompanied by binding of Ca^{2+} ions. As a prerequisite, centrins need to be activated by bound Ca^{2+} for the interaction with most of its known interacting partners (details see in Sections 3.1 and 5) (Schiebel and Bornens, 1995; Wiech et al., 1996; Durussel et al., 2000; Pulvermüller et al., 2002; Gießl et al., 2004b, 2006; Hu et al., 2004; Cox et al., 2005). In green algae, centrins serve as Ca^{2+} sensors at the contractile flagellar rootlets by recognizing the increase of the intracellular Ca^{2+} concentration (Sanders and Salisbury, 1989, 1994; Schiebel and Bornens, 1995). This binding of Ca^{2+} leads to an ATP-independent contraction of Ca affinity centrin-containing fibres of the flagellar rootlets (Sanders and Salisbury, 1994; Schiebel and Bornens, 1995).

Although the four EF-hand motifs of centrins are highly conserved, the Ca^{2+} affinities between the different EF-hands in centrins are not identical. In the green algae *Chlamydomonas*, centrin molecules bind two Ca^{2+} ions with high affinity at the N-terminal domain and two with low affinity at the C-terminal domain (Weber et al., 1994; Durussel et al., 2000). The N-terminus of *Chlamydomonas* centrin serves as a Ca^{2+} sensor and strengthens protein–protein interactions, like the complex formation with Sfi1p (Sheehan et al., 2006). In contrast to *Chlamydomonas* centrin where all EF-hands bind Ca^{2+}, in higher eukaryotic cells some EF-hand motifs lost the ability to bind Ca^{2+}. In HsCen2p, for example, Ca^{2+}-binding is only mediated via the EF-hand motif IV in the C-terminal domain (Durussel et al., 2000). This Ca^{2+}-binding induces conformational changes which lead to the exposure of hydrophobic surfaces and therefore supporting the formation of homodimers (Durussel et al., 2000; Tourbez et al., 2004). This functional EF-hand IV of HsCen2p is strictly Ca^{2+}-specific and does not bind other cations like Mg^{2+} (Durussel et al., 2000; Cox et al., 2005). Such mixed cation-binding sites were identified in HsCen3p and indicate isoform-specific regulations of the EF-hands. HsCen3p belongs to the second subfamily of centrins, related to yeast Cdc31p, and contains three functional EF-hands (Cox et al., 2005). However, two of them show only low Ca^{2+} affinity and one has a rather unspecific, but high affinity to both cations Ca^{2+} and Mg^{2+} (Cox et al., 2005). Preliminary data on MmCen1p indicate extraordinary high Ca^{2+} affinity of EF-hands in the C-terminal domain of the molecule (~1000 times higher than human calmodulin) (Black et al., 2006; Park et al., 2006). Although all centrins contain the same distribution of EF-hand motifs the regulation is highly selective since the binding properties differ between species and between isoforms in one species. Due to the fact that most known homomeric and heteromeric protein–protein interactions of centrins are Ca^{2+}-triggered, the Ca^{2+}-binding represents the most important, but not the only, molecular regulatory mechanism of centrins.

2.2.2. Site-specific phosphorylation of centrins

Besides Ca^{2+}-binding, phosphorylation represents a second principle modification of centrin molecules which regulates the functions of centrins in yeast, green algae and in mammalian cells (Salisbury et al., 1984; Martindale and Salisbury, 1990; Salisbury, 1995; Lutz et al., 2001; Gießl et al., 2004b; Trojan et al., 2008). In unicellular green algae, centrins are the major components of contractile fibres at the basal bodies. These fibres contract upon an increase of intracellular Ca^{2+}. For fibre relaxation, centrins have to be phosphorylated at the C-terminal domain (Salisbury et al., 1984; Martindale and Salisbury, 1990). In the green algae *Chlamydomonas*, protein kinase A (PKA) phosphorylates a centrin species at Ser167 *in vitro* (Meyn et al., 2006). The identified target sequence is located in the C-terminus, the highest conserved region of centrins. In human HeLa cells, phosphorylation of this conserved PKA phosphorylation site occurs in HsCen2p during the cell cycle at the G1/S transition (Lutz et al., 2001). Interestingly, centrins are hyperphosphorylated during the abnormal cell cycle of breast cancer cells obtained from human patients (Lingle et al., 1998).

Recently, we have identified CK2 as the protein kinase which phosphorylates murine centrin isoforms with high specificity in fully differentiated retinal photoreceptor cells (for details see Section 5.2) (Trojan et al., 2008). CK2 phosphorylation is centrin isoform specific. Only Cen1p, 2p and 4p, but not Cen3p are phosphorylated by CK2 at a specific site. Furthermore, CK2-mediated phosphorylation occurs in the dark and strongly reduces the binding affinities of centrins for target interactor proteins like G-proteins. The latter effect is probably due to the reduction of centrin Ca^{2+} affinity induced by phosphorylation.

In conclusion, regulatory modifications of centrins include two major events: Ca^{2+}-binding on the one hand and phosphorylation on the other hand. Comparisons of high-resolution structures in centrins under different Ca^{2+} concentration and phosphorylation states may provide more insights in these mechanisms.

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Table 2
Domain and full-length centrin structures from different sources determined by either NMR spectroscopy or X-ray crystallography

Structure	Reference	Method	PDB code	Deposition
C-HsCen2p[a]	Matei et al. (2003)	NMR	1M39	2003
C-CrCenp/Kar1p[b]	Hu and Chazin (2003)	NMR	1OQP	2003
C-HsCen2p/XPCp[c]	Yang et al. (2006a)	NMR	2A4J	2005
N-CrCenp[d]	Sheehan et al. (2006)	NMR	2AMI	2006
N-HsCen2p	Yang et al. (2006b)	NMR	1ZMZ	2006
HsCen2p/XPCp	Thompson et al. (2006)	X-ray	2GGM	2006
Cdc31p/Sfi1p[e] (Ca^{2+} bound)	Li, S. et al. (2006)	X-ray	2DOQ	2006
Cdc31p/Sfi1p (Ca^{2+} free)	Li, S. et al. (2006)	X-ray	2GV5	2006
HsCen2p/XPCp	Charbonnier et al. (2007)	X-ray	2OBH	2007
MmCen1p-L[f]	Park and Pulvermüller (unpublished)	X-ray	–	–

[a]'C-' indicates the C-terminal half of a centrin containing EF-hand motifs III and IV.
[b]Kar1p is a peptide derived from the cell division control protein Kar1p.
[c]XPC is a peptide derived from *Xeroderma pigmentosum* group C protein.
[d]'N-' indicates the N-terminal half of a centrin containing EF-hand motifs I and II.
[e]Sfi1p is a protein from the half bridge attached to the spindle pole body.
[f]MmCen1p-L is an N-terminally extended mouse Cen1p (Park et al., 2006, 2005).

2.3. *High-resolution molecular structure of centrins and their complexes*

Detailed protein structures at high-resolution based on nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography provide more insights into the molecular composition and function of molecules. High-resolution structural data on centrins from different organisms are available from solution studies by NMR spectroscopy and from X-ray crystallography (Table 2). These studies were carried out either on individual C- or N-terminal domains, or on full-length proteins, and the proteins were either in their unbound states or in complexes with target proteins.

As a result of these analyses, centrins are known to form dumbbell-like structures, which closely resemble those of the other proteins belonging to the calmodulin-parvalbumin superfamily (Fig. 2D). Pairs of the first and second EF-hands and of the third and the fourth EF-hands form compact structures in the N- and C-terminal halves, respectively, which are connected by a long central α-helix (Fig. 2D). Typically an 'open' conformation is reported for the C-terminal domains and a 'closed' conformation is found for the N-terminal domains of the centrin molecules. 'Closed' conformations are usually adopted by the Ca^{2+}-free domains of calmodulin-parvalbumin-type proteins, whereas Ca^{2+}-binding triggers a switch to the 'open' conformation as was shown in detail for troponin C by X-ray crystallography (Herzberg and James, 1985; Sundaralingam et al., 1985; Satyshur et al., 1994; Houdusse et al., 1997; Strynadka et al., 1997). Correspondingly, in the two crystal structures of the HsCen2p–XCPp complex (Table 2; for functional details see Section 5), the N-terminal domains are Ca^{2+}-free and each of the EF-hand motifs III and IV in the C-terminal domain has a Ca^{2+} ion bound (Thompson et al., 2006; Charbonnier et al., 2007). In agreement with a relatively low Ca^{2+} affinity of EF-hand III this site is occupied by Ca^{2+} in only 70% of the HsCen2p molecules in the crystal (Charbonnier et al., 2007). Surprisingly, a typical 'open' conformation is reported for the C-terminal domains of centrin molecules even in the Ca^{2+}-free Cdc31p/Sfi1p complex. In contrast, in the Ca^{2+}-bound Cdc31p/Sfi1p complex and in MmCen1p-L[2] Ca^{2+} ions are bound to the EF-hand I and both centrins adopt a 'closed' conformation (Li, S. et al., 2006; Park et al., 2006). Assuming that the Ca^{2+} affinities of the N-terminal domains of HsCen2p (Yang et al., 2006a) and Cdc31p are comparably low, the observed binding of Ca^{2+} to EF-hand I in one of the Cdc31p/Sfi1p complex structures is a result of the unphysiologically high Ca^{2+} concentration (0.1 M) used for crystallization (Li, S. et al., 2006). The fact that Ca^{2+} binding does not change the conformation was also shown by NMR spectroscopy for the N-terminal domain of HsCen2p (Yang et al., 2006a), but, on a structural level, the reason for this unusual behaviour of an EF-hand protein remains to be explained. Clearly different from the majority of centrins, which have been structurally investigated, the N-terminal domain of CrCenp adopts an 'open' conformation in the presence of Ca^{2+} and is proposed to act as a Ca^{2+} sensor (Sheehan et al., 2006).

Analyses of polymerization properties of centrins indicate that their Ca^{2+}-induced polymerization is mainly dependent on the N-terminal subdomain (Wiech et al., 1996). Studies on HsCen2p suggest that polymerization is facilitated by intermolecular interactions of the N- and the C-terminal subdomains (Yang et al., 2006a). NMR spectroscopy revealed the N-terminal subdomains to be of irregular and dynamic structure in solution (Yang et al., 2006a). These regions were also not visible in the electron

[2]N-terminally extended MmCen1p containing the additional GSPGISGGGGGIRLRAPLRSQLLWR peptide sequence.

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density maps of centrin crystals (Li, S. et al., 2006; Park et al., 2006; Thompson et al., 2006).

Taken together, the X-ray crystal structures of centrins available to date do not indicate that these proteins undergo substantial conformational changes upon Ca^{2+}-binding. This is in contrast to earlier observations made by circular dichroism-spectrometry (Wiech et al., 1996). However, the certain Ca^{2+} dependency of centrin functions might be related to subtle effects of submolecular structural rearrangements, modulation of flexibility or reduction of conformational heterogeneity resulting from Ca^{2+}-binding (Matei et al., 2003; Yang et al., 2006a; Charbonnier et al., 2007). Nevertheless, the reason for this unusual behaviour of an EF-hand protein remains to be explained on a structural level.

3. Subcellular localization and cellular function of centrins

3.1. Centrins as ubiquitous components of centrosomes, spindle poles and basal bodies

The first centrin protein was described as the major component of the massive striated flagellar rootlets of *Tetraselmis striata* (Salisbury et al., 1984). In these unicellular green algae, centrins-containing striated rootlets originate at the basal body apparatus, project into the cell body and extend to the plasma membrane, the nucleus or other organelles (Salisbury, 1989). Subsequently, centrin-based fibre systems were also described in several other green algae including the algal model system, the "green yeast" *Chlamydomonas rheinhardtii*. In *Chlamydomonas*, centrins are found in descending fibres which connect the basal body apparatus with the nucleus (Salisbury et al., 1987; Schulze et al., 1987), in distal fibres which connect both adjacent basal bodies to one another (McFadden et al., 1987) and in the stellate fibres of the transition zone present in the plane between the basal body and the axoneme of the flagella (Sanders and Salisbury, 1989) (Fig. 3A). The green algal centrin fibre systems exhibit Ca^{2+}-triggered contractions (Salisbury et al., 1984; Salisbury, 1995; Schiebel and Bornens, 1995). Contractions of these stellate fibres are thought to induce microtubule severing in the transition zone and thereby the excision of the flagellum (Sanders and Salisbury, 1989, 1994). Microtubule severing mediated by Ca^{2+}-activated centrin was discussed as a more wide spread phenomenon proceeding the massive reorganization of the microtubule cytoskeleton during cell migration (Salisbury, 1989) or contributing to the microtubule release from the centrosome, the major microtubule organizing centre (MTOC) of higher eukaryotic cells (Schatten, 1994). Nevertheless, the microtubule severing properties of centrin polymers are still under debate. There is reliable evidence that the AAA+ ATPases katanin may mediate axonemal severing during *Chlamydomonas* deflagellation (Lohret et al., 1998; Karabay et al., 2004; Baas et al., 2005).

However, centrins are not only found in nanofibres associated with cilia or basal bodies, but also as molecular components of the ciliary axonem and basal bodies (Huang et al., 1988a, b; Piperno et al., 1992; Baron et al., 1995; Guerra et al., 2003). There, centrins are required for basal body/centriole duplication which occurs during cell division (Geimer and Melkonian, 2004). Furthermore, the analysis of centrins in the free-living ciliate *Tetrahymena* provides evidence for a requirement of a centrin isoform for structural integrity of pre-existing centrioles (Stemm-Wolf et al., 2005).

In the yeast *Saccharomyces cerevesiae*, centrin is encoded by the *CDC31* gene. The yeast centrin orthologue protein, Cdc31p is a component of the half bridge of the spindle pole body (SBP) (Spang et al., 1993), the centrosome/spindle pole homologue in yeast (Fig. 3B). Cdc31p plays an essential role in the cell cycle via regulation of the duplication of the SBP in mitosis and meiosis II (Schiebel and Bornens, 1995; Geier et al., 1996; Wiech et al., 1996; Khalfan et al., 2000; Ivanovska and Rose, 2001). During the first steps of yeast SPB duplication, binding of Cdc31p to the protein Kar1p is required. In addition, Cdc31p specifically interacts with other yeast proteins including an essential protein kinase (Kic1p) whose activity probably regulates SPB duplication (Sullivan et al., 1998; Khalfan et al., 2000). Furthermore, during SPB duplication Cdc31p recruits Mps3p to the half bridge (Jaspersen et al., 2002). Mps3p is involved in sister-chromatid telomere cohesion (Antoniacci and Skibbens, 2006) and meiotic bouquet formation in *S. cerevisiae* (Conrad et al., 2007). Further observations indicated a role of Cdc31p in the nuclear mRNA export machinery in yeast (Fischer et al., 2004).

In vertebrates, centrin proteins are ubiquitously expressed and commonly associated with centrosome-related structures such as spindle poles of dividing cells or centrioles of centrosomes and basal bodies (Fig. 5) (Salisbury, 1995, 2007; Schiebel and Bornens, 1995; Wolfrum et al., 2002; Gießl et al., 2004b, 2006). As discussed above, the vertebrate centrins cluster to two divergent subgroups (Cen1p, 2p, 4p and Cdc31p/Cen3p) (Fig. 2A and B). This phylogenic and evolutionary diversity may also become manifest in differences in the cellular function and/or subcellular localization of the centrin isoforms. Unfortunately, little is known about the specific subcellular localizations and specialized functions of the different centrin isoforms and their specific function in diverse cell types and tissues. Many localization and biochemical studies in mammalian cells and tissues were performed with polyclonal and monoclonal antibodies generated against green algae centrins which do not discriminate between the mammalian centrin isoforms (e.g. Wolfrum and Salisbury, 1995, 1998; Laoukili et al., 2000; Pulvermüller et al., 2002; Gießl et al., 2004b). Unfortunately, such non-discriminatory antibodies are continually in use (Chang et al., 2006; Tsang et al., 2006) and are unhelpful to solve fundamental questions on specific molecular and cellular functions of centrin isoforms. Nevertheless, using

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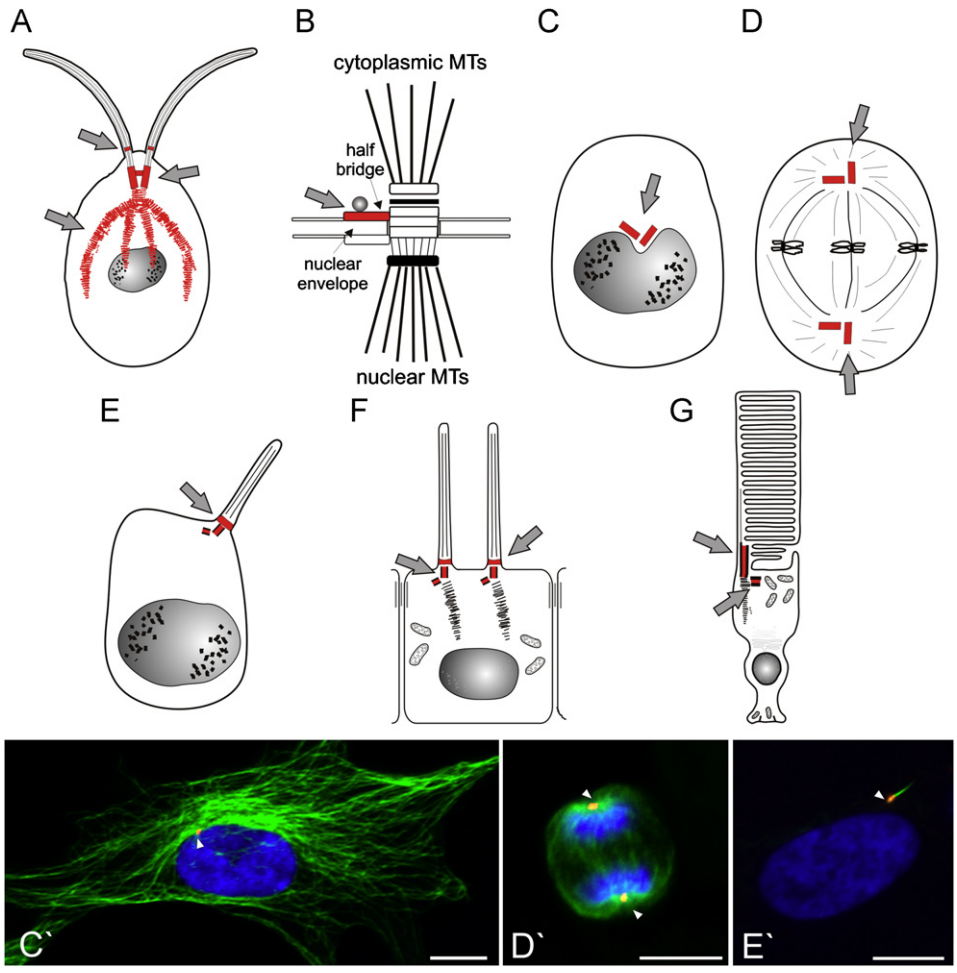

Fig. 3. Subcellular localization of centrins in different cell types. (A–G) Schematic representation of centrin localizations in various cell types is indicated in red and by arrows. (A) In unicellular green algae (e.g., *Chlamydomonas reinhardtii*), centrins are located at transition zones of flagella, basal bodies, distal connecting fibres and in nuclear-basal-body connectors. (B) The yeast centrin Cdc31p is localized at the half bridge of the spindle pole body, the major microtubule organizing centre (MTOC) in yeast. (C–E) Centrin localization in different stages of cultured animal cells. Centrins are localized in centrioles of centrosomes during interphase (C), the spindle poles in mitotic cells (D) and in the transition zone and in basal bodies of primary cilia in cell cycle-arrested cells (E). (F) In multi-ciliated epithelial cells, centrins are found in the transition zone and in basal bodies of cilia. (G) In the vertebrate photoreceptor cell, centrins are located in the connecting cilium (= elongated transition zone) and in the basal bodies of the ciliary apparatus. (C′–E′) Subcellular localization of centrins in cultured hTERT-RPE1 cells. Double immunofluorescence staining with antibodies against centrin isoform 3 (red) and anti-α-tubulin (green) of cells during interphase (C′) and in mitosis (D′). Cen3p is localized at the centrosome (*arrowhead*) during interphase (C′) and at the mitotic spindles of dividing cells (D′). (E′) Double immunofluorescence staining of anti-Cen3p (red) and acetylated-tubulin (green) in primary cilia of serum starved cells. Cen3p is exclusively located at the base of the primary cilium, the transition zone and the basal body (*arrowhead*). In contrast, acetylated-tubulin is found in microtubules throughout the entire length of the primary cilium. Nuclear DNA is counterstained with DAPI (4′,6-diamididino-2-phenylindole). Bars: 6 μm.

these antibodies, centrins were detected in centrioles and the pericentriolar matrix (PCM) of centrosomes and basal bodies (Salisbury et al., 1988; Baron et al., 1991, 1994). Furthermore, centrin was found to be localized in pericentriolar satellites which are interconnected and linked to the PCM by a network of centrin-containing nanofibres (Baron et al., 1992, 1994) and, in stably transfected cultured cells, green fluorescent protein (GFP) tagged-Cen1p was identified among the first proteins to localize at sites of newly forming centrioles (La Terra et al., 2005).

So far only a few studies have been performed with isoform-specific probes for mammalian centrin isoforms (Wolfrum and Salisbury, 1998; Salisbury et al., 2002; Gavet et al., 2003; Gießl et al., 2004a). In these analyses, centrin isoform-specific primers were applied in comparative combined reverse transcriptase reaction and polymerase chain reaction (RT-PCR) experiments and/or isoform-specific antibodies were successfully used. In summary, these expression analyses provide the following view: centrin isoforms Cen2p and 3p are ubiquitously expressed in all somatic cells; Cen1p is expressed in male germ cells and ciliated cells; Cen4p expression is restricted to ependymal and choroidal ciliated cells of the brain and ciliated sensory cells (Wolfrum and Salisbury, 1998; Laoukili et al., 2000; Gavet et al., 2003; Trojan, 2003; Gießl et al., 2004a). Cen2p and 3p are localized in the proximal portion of the centrioles in centrosomes, in the basal bodies of cilia and flagella as well as in the periciliary

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matrix surrounding the centrioles (Paoletti et al., 1996; Laoukili et al., 2000; Gießl et al., 2004a). In the course of mitosis, both, Cen2p and 3p, appear and stay in the spindle poles. In contrast to Cen2p and 3p, Cen1p was mapped to the transition zone of cilia and Cen4p was found in the basal body of ciliated neurons and sensory cells (Gavet et al., 2003; Gießl et al., 2004a).

The prominent localization of centrins at the centrosomes and basal bodies gave rise to several hypotheses regarding the cellular functions of centrins. In animal interphase cells or in arrested cells of differentiated tissue, the centrosome functions as the major MTOC (Fig. 3) (Bettencourt-Dias and Glover, 2007). At the MTOC, microtubules are *de novo* synthesized; the number and polarity of cytoplasmic microtubules is determined. It has been suggested that centrins are involved in the microtubule severing which should occur to release *de novo* synthesized microtubules from the pericentriolar origin (Schatten, 1994). However, more reliable evidence was gathered for important, but probably distinct roles of centrins at the centrosome during the cell cycle. The centrosome is duplicated once during the cell cycle to give rise to two spindle poles that organize the microtubule array of the mitotic spindle (Fig. 3D and D′). Like its close relative, the yeast Cdc31p, Cen3p may participate in centrosome reproduction and duplication during G2 of the interphase of mitosis (Middendorp et al., 2000). Cen2p seems to play a specific role in centriole separation preceding centrosome duplication (Lutz et al., 2001). Gene silencing experiments using RNA interference in human HeLa cells confirmed the requirement of Cen2p for correct centrosome duplication and for proper cytokinesis (Salisbury et al., 2002).

Nevertheless, centrins are not only expressed in the centrioles during the *de novo* formation of basal bodies or during the prearrangement and execution of centriol duplication, but also at basal bodies and centrioles and centrosomes in interphase G1 or in fully differentiated cells (G0) (Fig. 3E and E′). However, little is known about the function of centrins in the latter cell stages. In G1 and G0 cells, vertebrate centrins are probably required for structural integrity of pre-existing centrioles as shown for centrins in the ciliate *Tetrahymena* (Stemm-Wolf et al., 2005; Salisbury, 2007). Furthermore, centrins may contribute to membrane-independent G-protein signalling at the centrosomes and the basal body apparatus of ciliated cells (Gießl et al., 2004b). In the highly specialized, fully differentiated photoreceptor cells of the vertebrate retina binding of centrins to the visual G-protein may regulate the translocation of transducin through the connecting cilium (see below, Section 5.1).

4. Centrins in the vertebrate retina

4.1. Centrin expression in the vertebrate retina

Comparative studies demonstrate expression of centrins in the retina of species distributed throughout the subphylum of vertebrates (Fig. 4) (Wolfrum and Salisbury, 1998; Wolfrum et al., 2002). In mammals, RT-PCR analyses with isoform-specific primers demonstrate expression of all four known mammalian centrin isoforms in the retina (Wolfrum and Salisbury, 1998; Trojan, 2003; Gießl et al., 2004a). The RT-PCR results were confirmed in protein expression studies by Western blot analyses using specific antibody probes for specific centrin isoforms (Gießl et al., 2004a).

4.2. Subcellular localization of centrins in retinal cells — in particular in photoreceptor cells

As in other cell types of animal tissues, centrins are common components of centrioles of the centrosomes and of basal body apparatuses in neurons of the vertebrate retina (Figs. 3 and 6) (Wolfrum and Salisbury, 1998; Wolfrum et al., 2002). Furthermore, centrins were found in the connecting cilium of rod and cone photoreceptor cells in all vertebrate species investigated so far (Fig. 4) (Wolfrum, 1992; Wolfrum and Salisbury, 1995, 1998; Schmitt and Wolfrum, 2001; Pulvermüller et al., 2002; Wolfrum et al., 2002; Gießl et al., 2004a, b, 2006). Our detailed analysis of the diverse centrins in the mouse retina revealed differential expression and subcellular distribution of centrin isoforms (Figs. 5 and 6) (Gießl et al., 2004a, b, 2006): Cen2p and 3p are expressed in all cell types of the retina and the associated cells of the retinal pigment epithelium. As in other cell types, Cen2p and 3p are localized at the centrosomes of non-photoreceptor retinal neurons. In rod and cone photoreceptor cells, subcellular localization of Cen2p and 3p is found in the basal body and the connecting cilium. In contrast, the expression of Cen1p and 4p in the retina is restricted to photoreceptor cells. Cen1p and 4p are localized in the connecting cilium or in the basal body of rod and cone photoreceptor cells, respectively (Fig. 6). In conclusion, it is worth noting that rod and cone photoreceptor cells of the mammalian retina are the only cell types known so far that express all four centrins in parallel; three isoforms (Cen1p–3p) in the cilium and three in the basal body (Cen2p–4p) (Fig. 6).

High-resolution immunofluorescence techniques and immunoelectron microscopy enabled assignment of centrins to organelle substructures in retinal cells. As in centrioles of other cell types, centrins are found in the apical part of the centrioles. In the connecting cilium of photoreceptor cells, the ‘‘ciliary centrins’’, Cen1p–3p are localized along the entire extension of the connecting cilium (Figs. 5 and 6) (Wolfrum and Salisbury, 1995; Pulvermüller et al., 2002; Wolfrum et al., 2002; Gießl et al., 2004a, b, 2006). Therefore, antibodies raised against centrins are frequently used as molecular markers not only for centrioles (e.g. Nagasato and Motomura, 2004; La Terra et al., 2005; Dahm et al., 2007), but also for the connecting cilium (e.g. Liu et al., 1997; den Hollander et al., 2007; Overlack et al., 2008; Maerker et al., 2008). Immunoelectron microscopy data demonstrate that the

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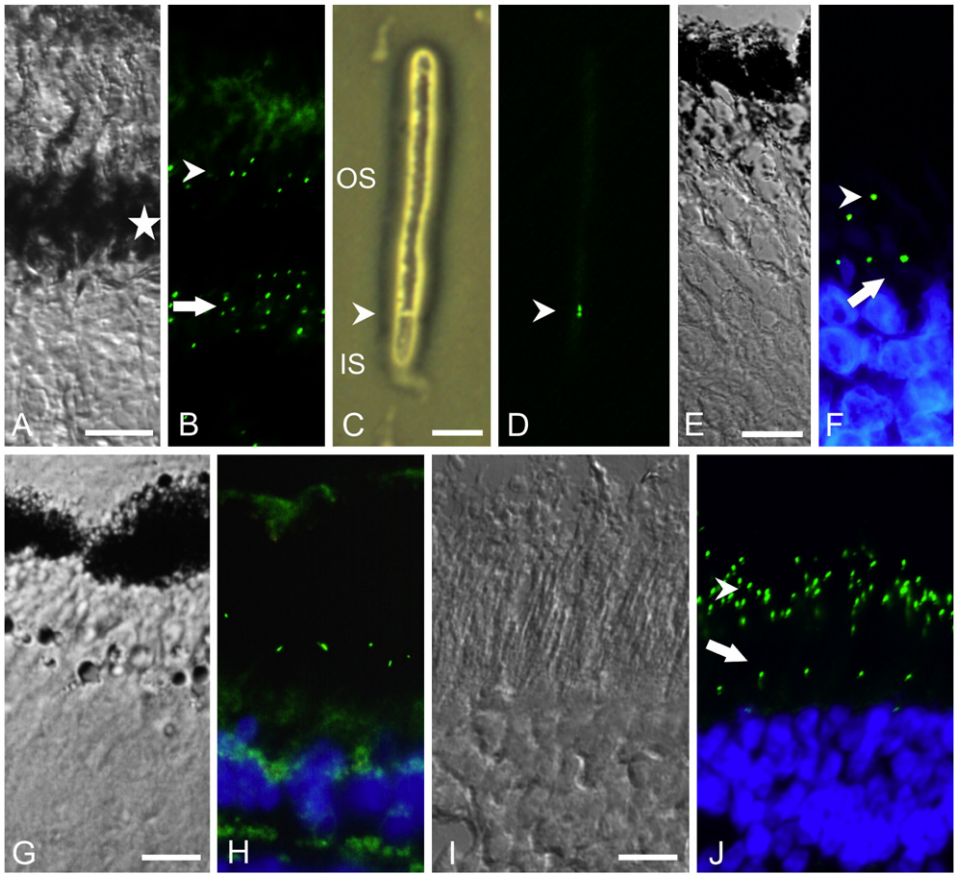

Fig. 4. Localization of centrins in vertebrate photoreceptor cells. (A–J) Immunofluorescence localizations of centrins in photoreceptor cells of species from different vertebrate classes. (A, C, E, G and I) Differential interference contrast images. (B, D, F, H and J) Indirect immunofluorescence staining with anti pan-centrin antibodies (green) and DAPI DNA staining (blue). Centrins are predominantly located in the ciliary apparatus, composed of the connecting cilium and the basal body. (A, B) Cryosection of a light-adapted fish retina (*Danio rerio*). Centrins are localized at the joint between the inner and outer segment of rods (*arrowhead*) and cones (*arrow*). Note: fish photoreceptor cells and RPE cells exhibit light-dependent retinomotor movement. In light-adapted retinas, melanin granules in the long microvilli-like extensions of the retinal pigment epithelium cells (*asterisk*) are located between the layer of rod and cone photoreceptor cells. (C, D) In an isolated rod photoreceptor cell of the teleost *Lepomis cyanellus*, centrins are stained in the short connecting cilium (green upper dot) and the basal body (green lower dot) (*arrowhead*). (E, F) In amphibian photoreceptor cells (*Ambystoma mexicanum*) centrin antibodies label connecting cilia in rods (*arrowhead*) and cones (*arrow*). (G, H) In chicken (*Gallus gallus*), pan-centrin antibodies label the ciliary apparatus of photoreceptor cells. (I, J) In the pig *Sus scrofa* (mammal), centrins are localized in the cilary apparatus of rod (*arrowhead*) and cone (*arrow*) photoreceptor cells.

"ciliary centrins" co-localize at the inner surface of the microtubule doublets of the connecting cilium (Fig. 5H; Wolfrum and Salisbury, 1998; Pulvermüller et al., 2002; U. Wolfrum and A. Gießl, unpublished data). Our recent observation of a direct binding of Cen1p to microtubules further supports this attachment to the ciliary microtubule pairs (Trojan et al., 2008).

The modified connecting cilium of vertebrate photoreceptor cells is the structural equivalent of an extended transition zone present at the base of a common motile cilium (Besharse and Horst, 1990; Liu et al., 2007; Roepman and Wolfrum, 2007). The presence of centrins along the entire extension of the connecting cilium is in agreement with the localization of centrins in the transition zone of motile cilia or the sensory cilia of mammalian olfactory cells (Wolfrum and Salisbury, 1998; Laoukili et al., 2000). The prominent localization of "ciliary centrins" in the connecting cilium certainly indicates a specific role of centrins in the function of the photoreceptor cilium. At the joint between the outer and the inner segment of the photoreceptor cell "ciliary centrins" may participate in the alignment of the photoreceptor outer segment (Wolfrum and Salisbury, 1995). In mammals, variation of the alignment angle of each outer segment is thought to achieve optimal light infiltration in each photoreceptor outer segment (Enoch, 1981). In addition, "ciliary centrins" may contribute to the massive molecular transport through the connecting cilium (Wolfrum and Salisbury, 1995). They may also contribute to the barrier for soluble proteins which is thought to be established in the connecting cilium for the regulation of molecular diffusion between the inner and the outer segment of photoreceptor cells (Spencer et al., 1988; Besharse and Horst, 1990; Wolfrum and Salisbury, 1998). In any case, centrin-based processes in the connecting cilium should be dependent on regulatory changes of the free Ca^{2+}

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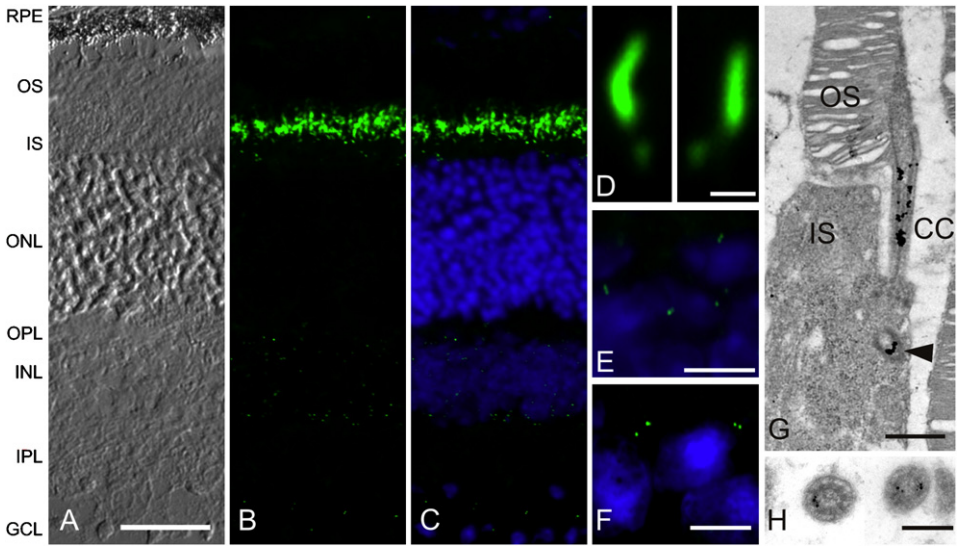

Fig. 5. Subcellular localization of centrins in the mouse retina. (A–F) Longitudinal cryosections through a murine retina. (A) Differential interference contrast image reveals the different layers of the retina. Light-sensitive outer segments (OS) are linked with inner segments (IS) of rod photoreceptor cells. Nuclei of photoreceptor cells are located in the outer nuclear layer (ONL), whereas synapses of photoreceptor cells and following neurons form the outer plexiform layer (OPL). Inner nuclear layer (INL) is composed of the nuclei of these neuronal cells. Their synapses in the inner plexiform layer (IPL) are linked to ganglion cells (GCL). RPE: retinal pigment epithelium. (B–F) Indirect immunofluorescence analysis of anti-pan-centrin. Centrins are mainly located in the ciliary apparatus, at the joint between the OS and the IS of photoreceptor cells (B–D). In addition, centrins are found at centrosomes in cells of the INL (B, C and E) and the GCL (B, C and F). (G and H) Immunoelectron microscopy analysis of ultrathin sections through parts of mouse rod photoreceptor cells. (G) Silver-enhanced immunogold labelling with antibodies specific for Cen3p reveals Cen3p localization in the connecting cilium (CC) and the basal body of a longitudinal sectioned rod cell. (H) Silver enhanced immunogold labelling of cross-sections through connecting cilia reveals localization of Cen3p at the inner surface of ciliary microtubule duplets. Bars: A–C: 20 μm; D and G: 0.5 μm; E and F: 4 μm; H: 150 nm.

concentration and/or the phosphorylation of centrin molecules (see Sections 2.2 and 5.2). Our recent results provide striking evidences for Ca^{2+}-dependent interaction between centrins and the visual G-protein transducin on its pathway through the inner lumen of the connecting cilium of mammalian photoreceptor cells (for details see Section 5.2) (Pulvermüller et al., 2002; Wolfrum et al., 2002; Gießl et al., 2004a).

5. Centrin-binding proteins in vertebrates

In the cellular context, proteins are in permanent crosstalk with interacting partner molecules throughout their entire molecular lifespan. Interacting molecules control, modify, protect proteins and guarantee the proper cellular function of the partners. Therefore, knowledge of specific interacting partners is a necessary prerequisite to solve the *in vivo* function of proteins. Unfortunately, in vertebrates, only few centrin-binding proteins are presently known. Numerous centrin-binding proteins have been genetically identified in yeast (see also Section 3.1). However, for most of theses proteins, which bind to Cdc31p, e.g. Kar1p, no vertebrate homologues have been identified so far.

In vertebrates, the first protein complexes containing centrins were isolated from the cytoplasm of arrested *Xenopus* oocytes. Centrins (XlCen2p and/or XlCen3p) are sequestrated in an inactive state by the interaction with the heat shock proteins HSP70 and HSP90 (Uzawa et al., 1995). In the mammalian retina, preliminary data indicate that HSP70/90 centrin complexes can also be assembled (U. Wolfrum, unpublished data).

In vertebrates, centrins were found in protein complexes containing centrosomal and ciliary proteins, e.g. CEP290 and CP110 (Chang et al., 2006; Tsang et al., 2006). CP110 and centrins were shown to interact not directly but functionally during cytokinesis (Tsang et al., 2006). The complex containing CEP290 and centrins is thought to be involved in the molecular transport through the connecting cilium of retinal photoreceptor cells and therefore being essential for photoreceptor maintenance and survival (Chang et al., 2006). Mutations of genes encoding molecules in this complex lead to several forms of photoreceptor degeneration and are causes for several forms of inherited blindness (Chang et al., 2006; Sayer et al., 2006). Furthermore, they are involved in Joubert syndrome, including retinal degeneration, *nephronophthisis* and cerebellar defects. Unfortunately, in these studies it remains elusive which centrin isoforms are integrated into the complexes containing CP110 and CEP290.

Proteins which interact directly with centrins were identified in yeast 2-hybrid screens (Paschke, 1997). Using human centrin 2 (HsCen2p) as a bait construct, the laminin-binding protein LBP, a component of the extracellular basal lamina, and the cytoplasmic receptor protein tyrosine kinase κ were identified as putative interaction partners (Paschke, 1997). To our knowledge, to date none of these putative centrin-binding proteins has an obvious

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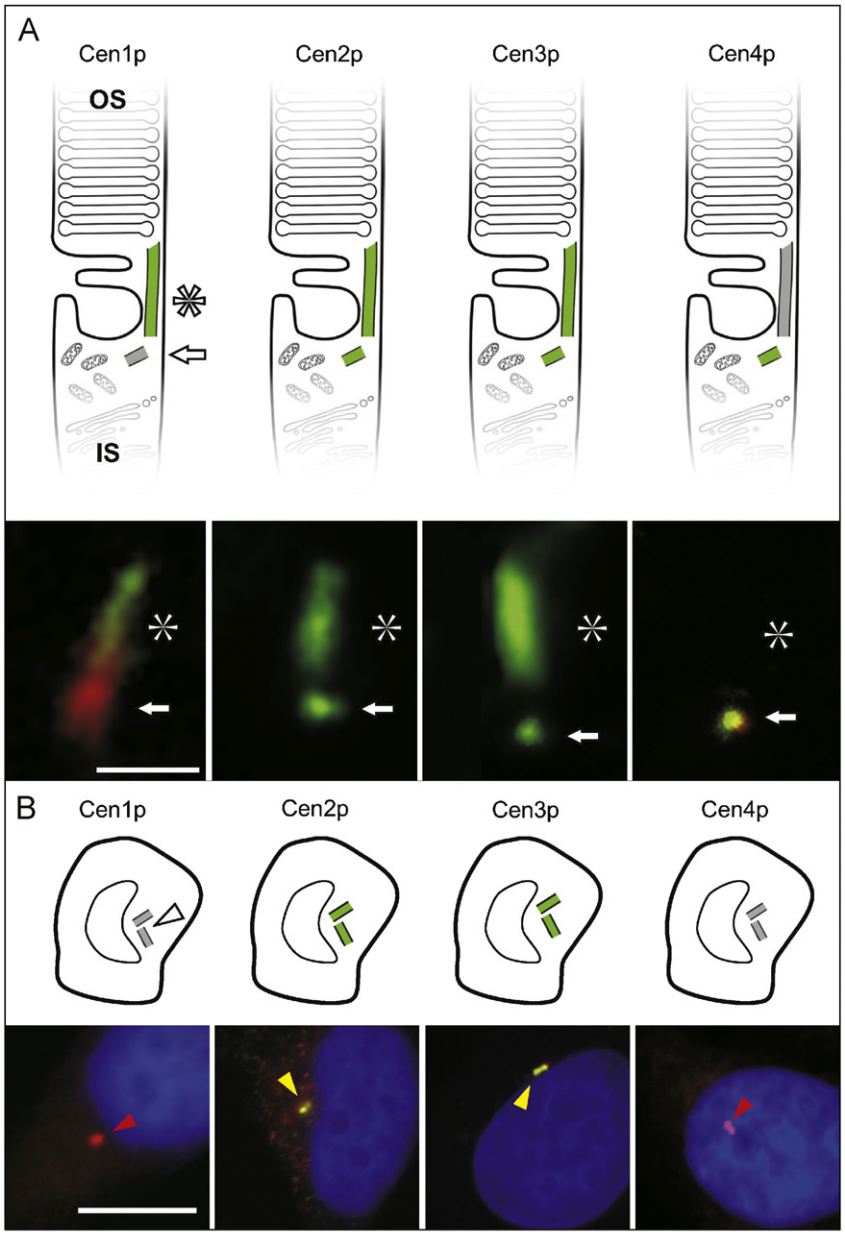

Fig. 6. Differential localization of centrin isoforms in the ciliary apparatus of vertebrate photoreceptor cells and at centrosomes of neuronal cells of the outer nuclear layer. (A) Schematic representation (upper part) and corresponding indirect immunofluorescence (lower part) of the isoform-specific localization of Cen1p–4p in the connecting cilium (*asterisk*) and at the basal body (*arrow*). Indirect immunofluorescence using centrin isoform-specific antibodies (green) with anti-γ-tubulin staining (red) reveal localization of Cen1p–3p in the connecting cilium. In addition, Cen2p and 3p are localized at the basal body, where Cen4p is exclusively localized and no Cen1p is detectable. (B) Cen2p and 3p-specific antibodies (green, *arrowhead*) react in dot pairs representing the centriole pairs in non-photoreceptor cells. DAPI stains the nuclear DNA (blue) (lower part). Schematic illustration of the differential localization of the four centrin isoforms in non-specialized eukaryotic cells (upper part). Cen2p and 3p are localized in centrioles of centrosomes, while no Cen1p and 4p is detectable (*arrowhead*). Bars: A: 1 μm; B: 8.2 μm.

function in the connecting cilium of mammalian photoreceptor cells. In cultured human HeLa cells, another interacting partner of HsCen2p was identified. HsCen2p was shown to interact directly with the *Xeroderma pigmentosum* group C protein (XPCp), which is responsible for nucleotide excision repair of genomic DNA (Araki et al., 2001; Charbonnier et al., 2006). The HsCen2p–XPCp complex seems to translocate from the centrosome and the cytoplasm into the nucleus after exposure of cells to UV-irradiation (Charbonnier et al., 2007). This process is thought to connect the functions of the centrosome and its integrative sensing with the XPCp-mediated DNA excision repair. Similar mechanisms are also conceivable for the function of centrins and XPCp in the photoreceptor cells to prevent damages after bright light irradiation.

A novel directly interacting partner of centrins was identified in yeast and termed Sfi1p (Kilmartin, 2003). Interestingly, Sfi1p was captured in a screen for

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Ca^{2+}-independent centrin interaction partners. This protein contains multiple conserved centrin-binding repeats and was discussed as a structural scaffolding protein forming Ca^{2+}-sensitive contractile fibres (Kilmartin, 2003; Li, X. et al., 2006). Mutations in SFI1 gene lead to drastic spindle pole defects in budding yeast indicating a role during the duplication of the MTOC and during mitotic spindle assembly (Kilmartin, 2003; Li, X. et al., 2006; Anderson et al., 2007). Sfi1p-like proteins containing centrin-binding repeats are conserved from yeast to humans (Kilmartin, 2003; Salisbury, 2004; Li, X. et al., 2006; Gogendeau et al., 2007). However, no clear evidence for an interaction of centrins with Sfi1p-like proteins and a functional role of such protein complexes has been described in higher eukaryotes so far.

5.1. Centrin-binding proteins in mammalian photoreceptor cells

Little is known about the expression and function of centrin-binding proteins like XPCp-protein or Sfi1p in the retina. Unpublished data obtained by RT-PCR indicate that Sfi1p is expressed in the murine retina (Ph. Trojan and U. Wolfrum, unpublished). Unfortunately, a further evaluation of the Sfi1p function in retinal photoreceptor cells is lacking.

A screen for centrin-interacting proteins in the retina by far Western blot analyses of retinal proteins overlayed with recombinant expressed MmCen1p revealed several polypeptide bands (Pulvermüller et al., 2002; Wolfrum et al., 2002). In these assays, binding of recombinant MmCen1p to target proteins was restricted to Ca^{2+}-activated centrin. An increase of affinity to a centrin target protein has been previously described for the binding of diverse centrin species to the yeast target protein Kar1p (Schiebel and Bornens, 1995; Geier et al., 1996; Wiech et al., 1996).

5.2. Molecular characteristics of centrin/transducin complexes

Analyses of proteins which were identified by our far Western blot screens of retinal centrin-binding proteins revealed the MmCen1p-binding protein p37 as the β-subunit of the visual G-protein transducin (G_t) (Fig. 7) (Pulvermüller et al., 2002; Wolfrum et al., 2002). Transducin (G_t) certainly plays a central role in the activation process of the visual signal transduction cascade in the vertebrate retina (Fung and Stryer, 1980; Heck and Hofmann, 1993, 2001) (see also Section 1). In recent years, we focused on the molecular and functional characterization of centrin/transducin complexes in photoreceptor cells of the mammalian retina.

In our initial studies, we demonstrated that Cen1p interacts with the visual G-protein transducin with high affinity, and thereby forms functional protein complexes in a Ca^{2+}-dependent manner (Fig. 7) (Pulvermüller et al., 2002; Wolfrum et al., 2002). Based on our knowledge of the differential expression of all four centrin isoforms (Cen1p–4p) in rodent photoreceptor cells (Gießl et al., 2004a, b), we addressed the question whether the Ca^{2+}-dependent assembly of centrin/transducin complexes also occurs between transducin and other centrin isoforms, namely Cen2p–4p. Applying independent but complementary interaction assays including co-immunoprecipitation,

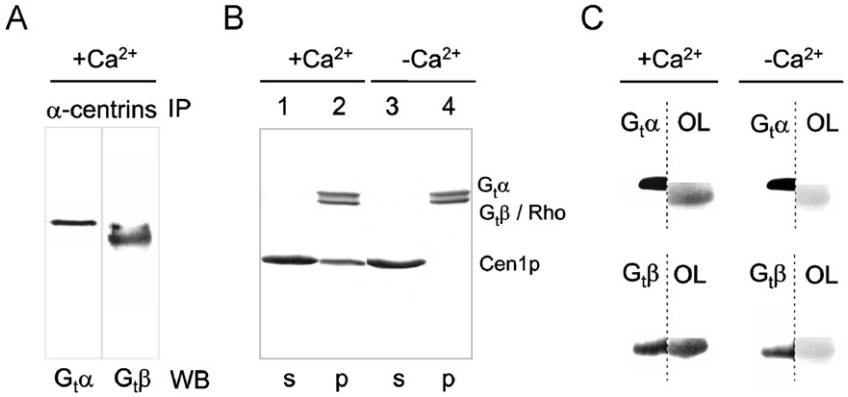

Fig. 7. Ca^{2+}-dependent assembly of MmCen1p/transducin complex. (A) Co-immunoprecipitation of transducin with Ca^{2+}-activated centrins from bovine retina lysates. Left lane: Western blot analysis with monoclonal antibodies against α-subunit of transducin ($G_t\alpha$) of an immunoprecipitation with monoclonal pan-centrin antibody (clone 20H5). Right lane: Western blot analysis with polyclonal antibodies against β-subunit of transducin ($G_t\beta$). The heterotrimeric G-protein complex (including $G_t\alpha/G_t\beta\gamma$) co-immunoprecipitates with MmCen1p in the presence of Ca^{2+}. (B) Membrane binding of the centrin/transducin complex. In the assay, centrin/transducin complexes are formed and bind to rhodopsin (Rh)-containing membranes of bovine photoreceptor cells. The pellets (p) and supernatants (s) were analysed using SDS-PAGE. (C) Combined far Western blot overlay analysis identifies retinal centrin-interacting protein p37 as $G_t\beta$ subunit of transducin. Left panel shows that in the presence of 1 mM $CaCl_2$, MmCen1p binds to a 37 kDa protein, which appears intensely labelled. Right panel shows that in the absence of Ca^{2+} (6 mM EGTA), MmCen1p binding is dramatically reduced. For specific determination of the centrin-binding protein Western blotted lanes were cut in half and processed in parallel for Western blotting with subunit-specific antibodies against $G_t\alpha$ (upper lanes), and $G_t\beta$ (lower lanes) and for overlays with recombinant expressed MmCen1p (OL). The 37 kDa centrin-binding protein is identified by centrin overlays and migrates in the probed SDS-PAGE at the exact mobility of the $G_t\beta$ subunit. (Adapted from Pulvermüller et al., 2002).

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GST-pull down, overlay and co-sedimentation assays as well as size exclusion chromatography and kinetic light-scattering experiments, we have shown that not only Cen1p, but also the three other centrin isoforms, Cen2p–4p, bind with high affinity to transducin (Pulvermüller et al., 2002; Gießl et al., 2004a, b, 2006). Further analyses using kinetic light-scattering experiments (see description in Fig. 8D) indicate that the centrin/transducin interactions are highly specific: centrin-related EF-hand proteins, calmodulin and recoverin, which are highly expressed in photoreceptor cells do not show any detectable Ca^{2+}-dependent interaction with transducin (Pulvermüller et al., 2002; Wolfrum et al., 2002; Gießl et al., 2004b). In addition, centrins do not bind to any other molecule of the visual transduction cascade, neither to arrestin, rhodopsin-kinase or rhodopsin, nor do they influence the activity of the cGMP PDE (Fig. 8) (Pulvermüller et al., 2002; Gießl et al., 2004b).

Analyses with our set of complementary protein–protein interaction assays further demonstrate that assembly of centrin/transducin complexes is mediated by the $\beta\gamma$-heterodimer (Fig. 7) (Pulvermüller et al., 2002; Wolfrum et al., 2002; Gießl et al., 2004a). Later studies also revealed a strict dependence of the assembly of these complexes on the free Ca^{2+} concentration. Titrations of the centrin isoforms in kinetic light-scattering experiments in the presence of Ca^{2+} showed differences in the affinity of the centrin isoforms to transducin. Cen3p has a significantly lower affinity to the transducin holoprotein than the other centrin isoforms (Gießl et al., 2004a). In the case of Cen1p and 2p, at least two Ca^{2+} ions are required for the activation of these centrin isoforms and for centrin/transducin complex formation (Pulvermüller et al., 2002; Trojan et al., 2008). Further analyses of these complexes indicate that Cen1p, 2p and 4p bind as homooligomers to the $G_t\beta\gamma$-heterodimer, in contrast to Cen3p which binds as

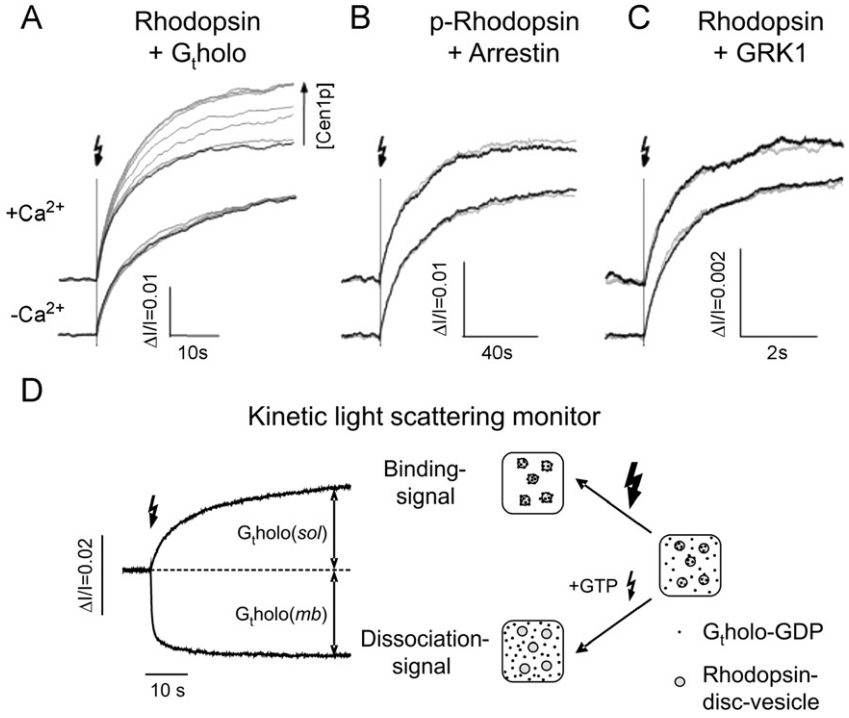

Fig. 8. Light-scattering setup to analyse the centrin/transducin assembly. (A) Ca^{2+}-dependent enhancement of kinetic light-scattering (KLS) binding signal with unphosphorylated membranes and transducin ($G_t$holo) in the presence of Cen1p. Upper panel represents KLS binding signals (3 μm rhodopsin, 0.5 μm $G_t$holo) in the presence of Ca^{2+}, and 0 (control, black curve), 0.6, 1.2, 2.5, 3.6, 5, 7.3, and 10 μm MmCen1p (grey curves), respectively. Lower panel represents KLS binding signals under the same conditions as in the upper panel, but with EGTA instead of Ca^{2+}. (B, C) KLS binding signal with unphosphorylated or prephosphorylated membranes and arrestin and rhodopsin kinase (GRK1) in the presence (grey curves) or absence (black curves) of MmCen1p. Upper panels represent KLS binding signals (0.5 μm arrestin or GRK1 and 3 μm rhodopsin) in the presence of Ca^{2+} plus/minus MmCen1p and the lower panels KLS binding signals in the absence of Ca^{2+}. Experimental conditions were 50 mM BTP, pH 7.5 containing 80 mM NaCl, 5 mM $MgCl_2$ and either 100 μm $CaCl_2$ or 1 mM EGTA at 20 °C, sample volume of 300 μl, and cuvette path length of 1 cm; 32% rhodopsin was photolyzed per flash (500 ± 20 nm). (D) Real-time monitoring of rhodopsin-transducin complex-formation by KLS. Shown is the time course of normalized light-scattering intensity originated from rhodopsin-containing disk vesicles. Left panel represents an example of KLS binding and dissociation signals. Reactions were triggered by flash photolysis of rhodopsin with a green (500 ± 20 nm) flash, attenuated by appropriate neutral density filters. The flash intensity is quantified photometrically by the amount of rhodopsin bleached and expressed as the mole fraction of photoexcited rhodopsin (Rh*/Rh). KLS binding signals (Rh*/Rh = 32%) were corrected by a reference signal (N-signal) measured on a sample without added protein as described by (Pulvermüller et al., 1993). KLS dissociation signals were recorded with a 0.5–5 ms dwell time of the A/D converter (Nicolet 400, Madison, WI) in the presence of 1 mM GTP and with catalytic amounts of flash activated rhodopsin (Rh*/Rh = 0.5%). To suppress base-line activation, 2.5 mM NH_2OH was added to the sample. The KLS binding signal is interpreted as a gain of protein mass bound to the disk membranes and the KLS dissociation signal as loss of protein mass from the disk vesicle (Heck et al., 2000). The right panel illustrates light-induced mass changes of the scattering membranous particles causing the binding and dissociation signal, respectively.

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a monomer to each $G_t\beta\gamma$ (Pulvermüller et al., 2002; Gießl et al., 2004a).

5.2.1. Functions of centrin/G-protein complexes in mammalian photoreceptor cells

At first view the spatial distribution of centrins (present in the connecting cilium and the basal bodies) and transducin (associated with the visual signal transduction machinery in the outer segment) should exclude any molecular interaction between centrins and transducin in photoreceptor cells. However, the visual G-protein transducin (G_t) is not a permanent resident of the outer segment. It undergoes light-dependent reversible bidirectional translocation in vertebrate photoreceptor cells (Fig. 9) (Brann and Cohen, 1987; Philp et al., 1987; Whelan and McGinnis, 1988). In the dark, G_t is highly concentrated in the rod outer segment, while under bright light conditions, the majority of G_t is translocated into the inner segment, the cell body, and even to the synapse of photoreceptor cells (Fig. 9) (Organisciak et al., 1991; Pulvermüller et al., 2002; Sokolov et al., 2002; Calvert et al., 2006). Interestingly, the clearance of transducin from the outer segment is completed in a few minutes after illumination and is much faster than transducin movements back into the outer segment (Sokolov et al., 2002; Calvert et al., 2006) indicating that different cellular mechanisms are involved (Peterson et al., 2003). Nevertheless, the bidirectional translocations of transducin are thought to contribute to slow but long lasting adaptation of rod photoreceptor cells (Sokolov et al., 2002, 2004; Hardie, 2003; Frechter and Minke, 2006).

Since any intracellular exchange between the inner and outer segmental compartments of photoreceptor cells occurs through the slender non-motile connecting cilium (Besharse and Horst, 1990; Roepman and Wolfrum, 2007) the bidirectional translocation of transducin should take this intracellular route (Fig. 9). Indeed, the power of immunoelectron microscopy provided us with the insight that transducin travels through the connecting cilium on its way between the two photoreceptor cell compartments (Pulvermüller et al., 2002). The slender, closely defined connecting cilium represents not only a track for the molecular translocation but also a suitable domain for regulation of intersegmental molecular exchange (Spencer et al., 1988; Besharse and Horst, 1990; Wolfrum and Salisbury, 1998), and the subcellular compartmentalization of the binding of transducin to the ciliary centrins.

As described in Section 4, centrins are present in the ciliary apparatus (connecting cilium plus basal body complex) of photoreceptor cells of all vertebrate retinas investigated. Our extensive analyses have shown that the four mammalian centrin isoforms (Cen1p–4p) are differentially localized in the ciliary apparatus of retinal photoreceptor cells (see Fig. 6) (Gießl et al., 2004a, 2006). These studies revealed the localization of the centrin isoforms Cen1p–3p in the connecting cilium of photoreceptor cells. Double-immunofluorescence analyses using

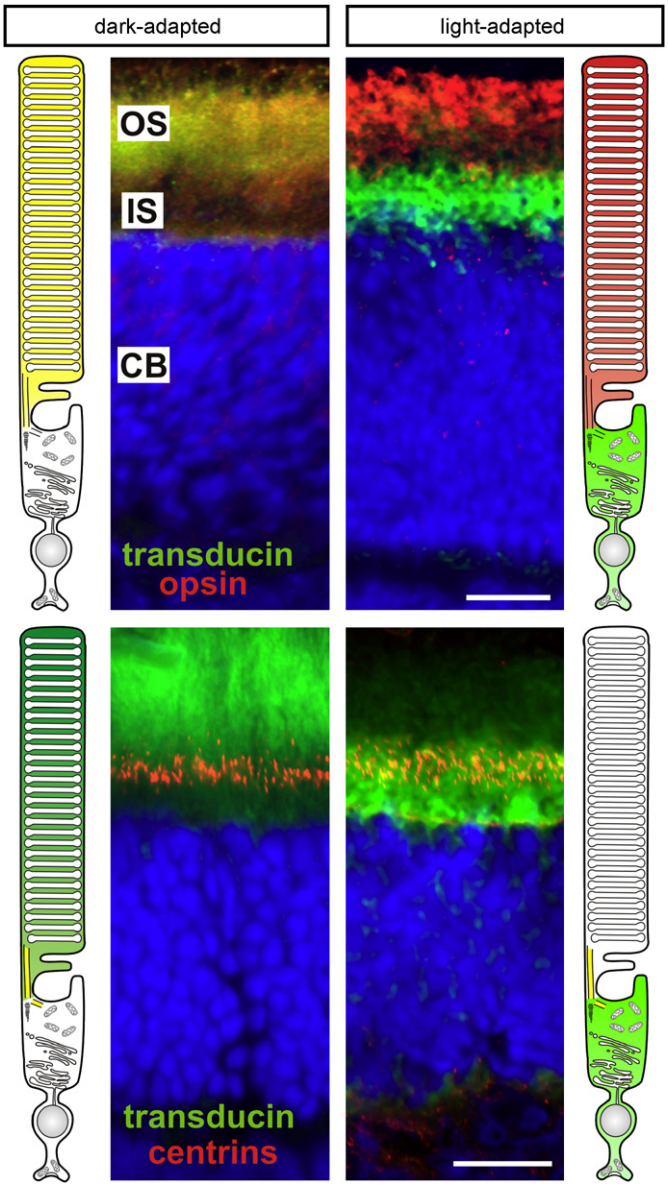

Fig. 9. Immunofluorescence localization of opsin, transducin and centrins in dark- and light-adapted mouse retinas. Upper panel: triple labelled dark-adapted (left) and light-adapted (right) mouse retina. Transducin (green) is localized to the outer segment (OS) of dark-adapted photoreceptor cells and moves into the inner segment (IS) and cell body (CB) during light adaptation. Opsin (red) stays in the outer segment during both conditions. Lower panel: Triple labelled dark-adapted (left) and light-adapted (right) mouse retina. Transducin (green) is localized as described for the upper panel. Centrins (red) are stained in the connecting cilium and basal bodies at the joint between the outer segment and the inner segment of dark- and light-adapted photoreceptor cells. Transducin passes centrins during its passage through the connecting cilium. Bars: 13.1 μm.

antibodies against centrins and transducin subunits show co-localization of the ciliary centrins in the photoreceptor connecting cilium (Pulvermüller et al., 2002; Wolfrum et al., 2002; Gießl et al., 2004a, b, 2006). Quantitative analyses of silver-enhanced immunogold labelling revealed that ciliary centrins and transducin share the same

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subciliary domain, at the inner surface of the microtubule doublets of the connecting cilium (Pulvermüller et al., 2002; Wolfrum et al., 2002; Gießl et al., 2004a). This spatial subciliary co-localization is consistent with the hypothesis that the ciliary centrins Cen1p–3p and transducin physically interact in the ciliary compartment of vertebrate photoreceptor cells. A changeover of assembly and disassembly of centrin/transducin complexes may regulate the diffusion of transducin through the connecting cilium.

5.2.1.1. Regulation of centrin/G-protein complex assembly in photoreceptor cells. As described above in Section 5.2.1, the binding of centrins to transducin is strictly dependent on Ca^{2+} activation of centrins. In photoreceptor cells, the activation of the ciliary centrins Cen1p and 2p should be triggered by an increase of the free Ca^{2+} concentration in the connecting cilium. There are several alternative mechanisms how the local intraciliary free Ca^{2+} concentration is modulated: Firstly, the intraciliary Ca^{2+} concentration may be affected by light-modulated changes of free Ca^{2+} in the outer segment. Light induces a decrease of Ca^{2+} in the outer segment which occurs within the operating (single quantum detective) range of the rod (Molday and Kaupp, 2000). This would cause an inactivation of centrins in the cilium upon outer segment illumination. However, recent observations also indicate a Ca^{2+} increase in bright light (rod saturated conditions) which would activate the ciliary centrins (Matthews and Fain, 2001; Leung et al., 2007). In any case, we cannot estimate which one of these opposite light-modulated changes in the free Ca^{2+} concentration in the outer segment may regulate the affinity of centrins to transducin. Finally, there is some indication for the presence of a local modulation of the Ca^{2+} homeostasis in the connecting cilium that is independent from the outer segment (Krizaj et al., 2002; Gallagher et al., 2006): Ca^{2+} fluxes through TRPP2 (polycystin-2) channels and the plasma membrane Ca^{2+}-ATPases (PMCA), recently identified in the connecting cilium, may modulate the intraciliary free Ca^{2+} homeostasis. Whatever process leads to an increase of the intraciliary Ca^{2+} concentration, Ca^{2+} activates the ciliary centrins Cen1p and 2p and promotes the assembly of centrin/G-protein complexes.

In the cilium, the Ca^{2+}-triggered assembly of centrin/G-protein complexes may contribute to a barrier for further exchange of transducin between the photoreceptor inner and outer segment (barrier hypothesis) (Wolfrum et al., 2002). A drop of free Ca^{2+} in the connecting cilium should induce the disassembly of the complex, thus providing the necessary condition for the exchange of transducin between the inner and the outer segment of photoreceptor cells described above (Philp et al., 1987; Whelan and McGinnis, 1988; Organisciak et al., 1991; Pulvermüller et al., 2002; Sokolov et al., 2002, 2004; Wolfrum et al., 2002; Mendez et al., 2003; McGinnis, 2004). However, Ca^{2+}-triggered sequential binding of transducin to centrins may contribute to the transport of transducin through the photoreceptor connecting cilium (Ca^{2+}-gradient hypothesis) (Wolfrum et al., 2002).

5.2.1.2. Regulation of centrin/G-protein complex assembly by centrin phosphorylation. Centrin functions are not only regulated by Ca^{2+}-binding but also by site-specific phosphorylation (see Section 2.2.1). This is certainly also the case in photoreceptor cells of the mature mammalian retina. Our most recently obtained data indicate antagonistic regulation of the formation of centrin/transducin complexes by Ca^{2+}-binding and CK2-mediated phosphorylation in retinal photoreceptor cells (Trojan et al., 2008). In *ex vivo* phosphorylation assays in explanted rat retinas, we found a drastic increase of centrin phosphorylation during dark-adaptation (Fig. 10A). This phosphorylation of centrins turned out to be highly specific for CK2, since it is completely inhibited by specific protein kinase CK2-inhibitors (Fig. 10A). This CK2-mediated phosphorylation of centrins is highly specific for the ciliary centrins Cen1p and 2p (Fig. 10B) (Trojan et al., 2008) and is abolished by dephosphorylation mediated by specific isoforms of the protein phosphatase PP2C (Thissen et al., in prep.). Furthermore, we provide evidence for a reduced Ca^{2+} affinity of phosphorylated centrins causing a reduction of their affinity to transducin (Trojan et al., 2008). In conclusion, CK2-mediated phosphorylation of centrins lowers the ability for the assembly of centrin/transducin complexes in the connecting cilium of dark-adapted photoreceptor cells. An extension of the previous displayed barrier hypothesis on the function of centrins in the connecting cilium of photoreceptor cells is shown in Fig. 11. The light-triggered signalling pathway that targets the antagonistic regulation of the centrin/transducin complex assembly remains elusive. The phosphorylation of centrins can be modulated via illumination either by the reduction of the CK2 activity or indirectly by an activation of PP2C in light. In our opinion, there is no doubt that the formation of centrin/transducin complexes is physiologically regulated and seems to play an important role for photoreceptor cell function.

6. Summary and conclusions

Centrins are members of a conserved subfamily of EF-hand Ca^{2+}-binding proteins commonly associated with the function of centrioles and centrosomes in the cell cycle. In the fully differentiated photoreceptor cells of the mammalian retina, four centrin isoforms are differentially expressed in the ciliary apparatus of photoreceptor cells. In the connecting cilium, ciliary centrins are localized at a subcellular compartment strategically favourable for the regulation of molecular exchange between the inner segment and the outer segment of photoreceptor cells. The unconventional search for centrin-binding proteins in the mammalian retina by far Western blot analyses resulting in identification of transducin as an interacting partner, together with complementary-independent

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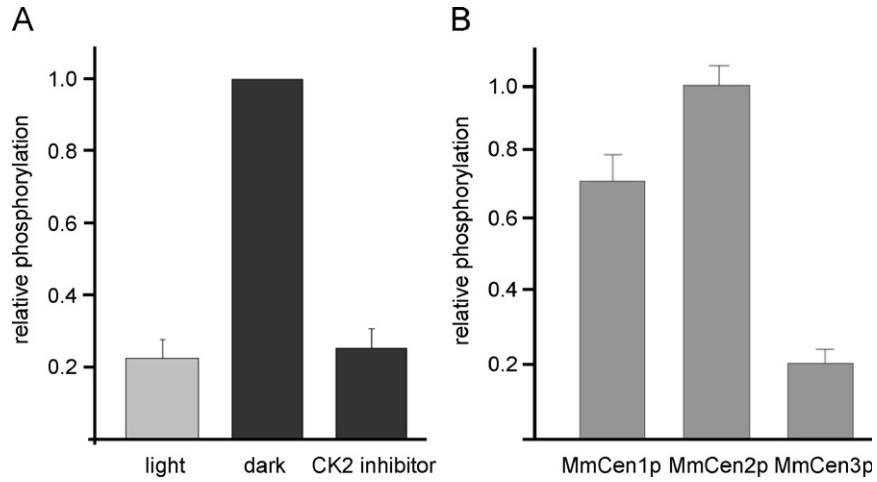

Fig. 10. *In vitro* and *ex vivo* phosphorylation of centrins. (A) Light-dependent *ex vivo* phosphorylation of endogenous centrins. Rats were light (grey bar) or dark (black bars) adapted. After sacrifice of the animals, the retinas were explanted and cultured in the presence of radioactive labelled phosphate. Centrins were immunoprecipitated from these retinas using a monoclonal pan-centrin antibody (clone 20H5) and the radioactive incorporation was analysed. Centrins were five times higher phosphorylated from dark-adapted retinas (dark) compared to centrins from light-adapted ones (light). This light-dependent phosphorylation could be inhibited by the specific protein kinase CK2-inhibitor 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole (DRB) in dark-adapted retinas. (B) CK2-mediated *in vitro* phosphorylation of ciliary centrins MmCen1p–3p. Recombinant MmCen1p–3p were phosphorylated by CK2 in the presence of radioactive ATP. Radioactive incorporation was analysed and the highest radioactive incorporation was set as 1 (MmCen2p). CK2-mediated phosphorylation is isoform specific, since only MmCen1p and 2p but not MmCen3p could be phosphorylated *in vitro*.

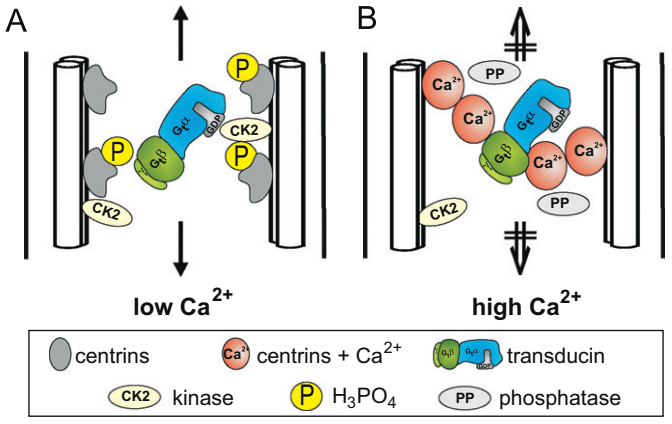

Fig. 11. Model on Ca^{2+}-dependent centrin/transducin complex assembly in the connecting cilium of vertebrate photoreceptor cells. (A) Under low free Ca^{2+} concentrations centrins are phosphorylated by protein kinase CK2 and not active, so transducin floats through the inner lumen of the connecting cilium. (B) If free Ca^{2+} increases in the cilium, centrins are dephosphorylated and activated by Ca^{2+} inducing the assembly of centrin/transducin complexes.

validation strategies supplies reasonable evidence for a specific function of centrins in photoreceptor cells. The molecular characterization of centrin/transducin complexes provides us valuable insights in the highly regulated interplay between the two partner proteins and their putative role in the regulation of light-dependent inter-compartmental exchange of the visual G-protein transducin. Moreover, a physiological relevant interaction between G-proteins and centrins can be judged as a novel aspect of the potential link of signal transduction and molecular translocation and should support insights into the supply of signalling proteins in general.

7. Future directions

Ongoing research utilizing improved isoform-specific antibodies to centrins and tagged-centrin constructs will provide more reliable information on the specific sub-cellular targeting and localization of centrin isoforms in their cellular environment. These results will not only provide better insights in photoreceptor function but also lead to a better understanding of centrins' role in the control of centriole duplication during the cell cycle in general. Since centrins additionally play an essential role in the basal body duplication during ciliogenesis and this aspect is crucial in differentiation of photoreceptor cells from progenitor cells, efforts to analyse the role of centrins during developmental stages of the retina will be very helpful. Further analyses of properties of the centrin/transducin complexes are currently being addressed to understand specific reciprocal-binding sites establishing the specific interaction between centrin isoforms and the $\beta\gamma$-subunit of the G-protein. High-resolution structure analyses of crystals of centrins in specific physiologic stages and centrin/transducin complexes co-crystals by NMR-spectroscopy and X-ray crystallography will help to elucidate the submolecular characteristics of centrin functions and the centrin/G-protein interaction. Future studies will be well worthwhile on the *in vivo* validation of current knowledge on centrin/transducin complexes, obtained by complementary and independent *in vitro* assays. Chasing further potential centrin-binding partners in the retina will certainly deepen our knowledge on the role of centrins in photoreceptor cell function. The establishment of knock out models deficient in centrin isoforms will provide valuable insights into the biological role of centrins.

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However, because of the fundamental roles of most centrin isoforms in the cell cycle, where they are essential for the proper embryonic development all known attempts of the generation of centrin knock out mice have failed due to lethal defects in early developmental stages. Ongoing research utilizing conditional knock out mice will overcome the latter problems. An alternative strategy that substitutes the time and resource-consuming knock out approaches would be the future application of RNAi technologies *in vivo* or *ex vivo* in organotypic retina cultures. For this purpose, improved gene transfer technologies for the application of centrin probes in the living animal or in organotypic retina cultures are currently being tested. Functional analysis of centrins in retinal photoreceptor cells will provide further insights in the cellular and molecular function of centrins in general and may also elucidate the role of transducin and heterotrimeric G-proteins at the centrosome of the eukaryotic cell.

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