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Insights into functional aspects of centrins from the structure of N-terminally extended mouse centrin 1

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Abstract

Centrins are members of the family of Ca^{2+}-binding EF-hand proteins. In photoreceptor cells, centrin isoform 1 is specifically localized in the non-motile cilium. This connecting cilium links the light-sensitive outer segment with the biosynthetic active inner segment of the photoreceptor cell. All intracellular exchanges between these compartments have to occur through this cilium. Three-dimensional structures of centrins from diverse organisms are known, showing that the EF-hand motifs of the N-terminal domains adopt closed conformations, while the C-terminal EF-hand motifs have open conformations. The crystal structure of an N-terminally extended mouse centrin 1 (MmCen1-L) resembles the overall structure of troponin C in its two Ca^{2+} bound form. Within the N-terminal extension in MmCen1-L, residues W24 and R25 bind to the C-terminal domain of centrin 1 in a target-protein-like geometry. Here, we discuss this binding mode in connection with putative interaction sites of the target-protein transducin and the self-assembly of centrins.

Keywords: Centrin; Calcium-binding protein; EF-hand; Connecting cilium; X-ray structure

1. Introduction

The photoreceptor cells of the vertebrate eye are highly specialized neurons which consist of morphologically and functionally distinct compartments; outer segment, inner segment (nucleus, synaptic terminal), and connecting cilium. The rod photoreceptor outer segment contains hundreds of membrane discs with the visual pigment rhodopsin, and many other proteins of the visual transduction cascade (Dowling, 1970). A slender cellular bridge, the so-called connecting cilium, links the outer segment with the inner segment, which contains the organelles typical for eukaryotic cells (Besharse & Horst, 1990). Centrin isoforms are differentially localized in the ciliary apparatus of the photoreceptor cells (Gießl et al., 2004a; Wolfrum, 1995). The localization of centrin isoforms 1–3 (Cen1, Cen2, and Cen3) is restricted to the inner lumen of the connecting cilium. Here, Cen1 and Cen2 participate in the regulation of the light-dependent bidirectional translocation of the visual G-protein, transducin (G_t), through the connecting cilium via Ca^{2+}-triggered binding to the $G_t\beta\gamma$ subunit complex of the heterotrimeric G-protein (Gießl et al., 2004a; Gießl, Trojan, Pulvermüller, & Wolfrum, 2006 in this issue of vision research; Wolfrum, Giessl, & Pulvermuller, 2002; Wolfrum et al., 2002).

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Centrins are relatively small acidic proteins that contain four helix–loop–helix motifs, the so-called EF-hands, which represent potential Ca^{2+}-binding sites (Gießl, Trojan, Pulvermüller, & Wolfrum, 2004b; Salisbury, 1995; Schiebel & Bornens, 1995; Wolfrum et al., 2002). These conserved sites specify them as members of a closely related subfamily within the large superfamily of Ca^{2+}-binding EF-hand proteins including calmodulin (CaM), troponin C (TnC), and parvalbumin (Kretsinger, 1976; Moncrief, Kretsinger, & Goldman, 1990; Nakayama, Moncrief, & Kretsinger, 1992). Four centrin proteins (Cen1, Cen2, Cen3, and Cen4) have been identified so far in mammals (Errabolu, Sanders, & Salisbury, 1994; Gavet, Alvarez, Gaspar, & Bornens, 2003; Lee & Huang, 1993; Levy, Lai, Remillard, Heintzelman, & Fulton, 1996; Madeddu, Klotz, Le Caer, & Beisson, 1996). Gene cloning studies of centrins have been applied for the identification of centrin genes in a variety of species from all kingdoms of eukaryotic organisms such as fungi, plants, and animals (Baum, Furlong, & Byers, 1986; Huang, Mengersen, & Lee, 1988; Zhu, Bloom, Lazarides, & Woods, 1995; Meng et al., 1996). Amino acid sequence comparisons revealed that Cen3 is closely related to the *Saccharomyces cerevisiae* homologue Cdc31, whereas Cen1 and Cen2 are more similar to *Chlamydomonas reinhardtii* centrin (CrCen) (Middendorp, Paoletti, Schiebel, & Bornens, 1997). Moreover isoforms, Cen1 and Cen2, are very similar showing amino acid identities of about 80–90% in vertebrates, whereas sequences of the yeast centrin and the related vertebrate Cen3 isoforms have amino acid identities of about 55% only, compared to the other isoforms. On the amino acid sequence level, Cen4 is closer to the subgroup of Cen1, Cen2, and CrCen than to the subgroup containing Cen3 and yeast centrin (Gavet et al., 2003; Gießl et al., 2004a).

Several functions of centrin have been discussed in diverse cells. First, in green algae, centrin fibers contract in response to an increase of the intracellular Ca^{2+}-concentration in the transition zone which is located between the flagellar axoneme and the basal body. The Ca^{2+}-triggered contraction of centrin fibers of the transition zone induces microtubule severing and thereby the excision of the flagellum (Sanders & Salisbury, 1994). Second, in baker's yeast *S. cerevisiae*, Cdc31 functions in the duplication of the spindle pole body. During the first step of the yeast spindle pole body duplication the binding of Cdc31 to Kar1 is required. Furthermore, Cdc31 specifically interacts with other yeast proteins including an essential kinase (Kic1) that probably regulates the spindle pole body duplication (Khalfan, Ivanovska, & Rose, 2000; Sullivan, Biggins, & Rose, 1998). Third, in invertebrates, centrins are ubiquitously expressed and commonly associated with centrosome-related structures such as spindle poles of dividing cells or centrioles in centrosomes and basal bodies (Salisbury, 1995; Schiebel & Bornens, 1995). It has been suggested that Cen3 participates in centrosome reproduction and duplication, while Cen1/Cen2 may play a role in centriole separation preceding centrosome duplication during the cell cycle (Gießl et al., 2004a; Salisbury, 2004).

Fourth, functions of centrins in vertebrate cells are also known. It has been reported that human centrins are a stabilizing component of xeroderma pigmentosum group C protein (XPC) and HRad23B complexes (Araki et al., 2001; Popescu et al., 2003). The XPC containing heterotrimer is involved in recognition of DNA lesions and initiation of global genome nucleotide excision repair. This is an important DNA repair pathway for damage caused by ultraviolet radiation, carcinogens, and chemotherapeutic agents, and impairment of XPC function is associated with the genetic disorder xeroderma pigmentosum. Human centrin 2 (HsCen2) appears to promote DNA binding by XPC both *in vivo* and *in vitro* and increases the specificity of the heterotrimer for damaged DNA (Nishi et al., 2005). The mechanism by which HsCen2 binds to XPC is still not understood. In highly specialized photoreceptor cells, Ca^{2+}-activated Cen1 and Cen2 bind with high affinity to G_t via its $G_t\beta\gamma$ (isoforms $\beta_1\gamma_1$) subunit complex and can thus regulate G_t translocation through the photoreceptor cilium (Gießl et al., 2004a, 2004b; Pulvermüller et al., 2002; Wolfrum et al., 2002).

Here, we discuss the structure of N-terminally extended mouse centrin 1 (MmCen1-L) in comparison with the already known 3D structures of full-length centrin as well as those of N- and C-terminal domains.

2. Structures of centrins

Centrins from diverse species have relatively high sequence homologies (55–85%). Several solution NMR structures of centrin domains as well as a crystal structure of full-length centrin have been reported: the X-ray structure of the human full-length HsCen2 in complex with a peptide derived from the XPC protein (Thompson, Ryan, Salisbury, & Kumar, 2006), NMR structures of the C-terminal domain of HsCen2 in complex with the same XPC-derived peptide (Yang et al., 2006a) and in its unbound state (Matei et al., 2003), the N-terminal domain of HsCen2 (Yang et al., 2006b), the N-terminal domain of CrCen (Sheehan et al., 2006) and the C-terminal domain of CrCen complexed with a peptide derived from the protein Kar1 (Hu & Chazin, 2003). Sequence alignments of centrins with known structures show fairly high homology to mouse centrin 1 (MmCen1; Fig. 1). In all centrin structures determined so far, the N- and C-terminal domains resemble the corresponding domains of troponin C or calmodulin.

We crystallized MmCen1-L, an N-terminally extended version, as described earlier (Park et al., 2005) and determined the structure at 1.8 Å resolution (to be published elsewhere). MmCen1-L contains 25 additional amino acid residues upstream of the N-terminus of the wild-type MmCen1 sequence. The refined structural model of MmCen1-L contains W24 and R25, which represent the last two amino acids of the N-terminal extension preceding the original start codon of wild-type MmCen1, and residues D53 to K192 out of the 197 residues which constitute MmCen1-L. Superposition of the structures of MmCen1-L