

Figure 1: Structure of human CaM. CaM contains a pair of EF-hand with Ca²⁺ (yellow) in N-terminus and C-terminus (PDB code: 1CLL) [13].

Centrin is composed of two independent domains, each of which contains a pair of EF-hand motifs (Figure 2). Figure 2 shows the sequence alignment of centrin from various organisms. The C-terminal domain of centrin is highly conserved. The Ca²⁺ loops are composed of acidic residues, which bind Ca²⁺. However, loop I and loop II which are located in the N-terminal do not bind Ca²⁺ conversely loop III (D114-N125) and loop IV (D150-E161) which are located in the C-terminal bind Ca²⁺.

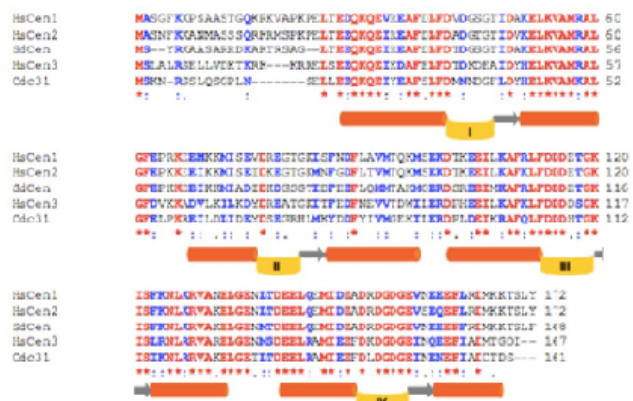


Figure 2: Centrin sequences with EF-hand motifs. Centrin alignment showing the four EF-hand loops of *Saccharomyces cerevisiae*, *Scherffelia dubia* and human centrin.

Figure 3 shows the structure of human centrin 2 [14]. The Ca²⁺ site of the loop III consists of five oxygen atoms belonging to the side-chains of residues D114, D116, T118, and N125 and the backbone of K120 of the protein and by a sixth oxygen from a water molecule. The Ca²⁺ site of the loop IV consists of six oxygen atoms belonging to the side-chain of residues D150, D152, D154, E161 or the backbone E156 of the protein and by a seventh oxygen from a water molecule. NMR structures of the C-terminal domain of human centrin 2 revealed that Ca²⁺ binding modifies the conformation of this protein [15].

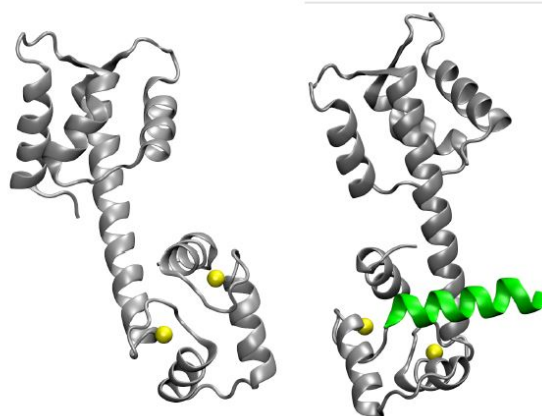


Figure 3: Structure of human centrin 2 and human centrin 2 in complex with XPC peptide. Structure of centrin 2 showing Ca²⁺ bound to a pair of EF-hand in C-terminus (left). Structure of centrin in complex with XPC peptide (right) (PDB code: 2GGM) [31].

Protein	EF-hand motif
CaM	
Loop I	21-DKDGNGTITTKE-32
Loop II	57-DADGNGTIDFPE-68
Loop III	94-DKDGNGYISAAE-105
Loop IV	130-DIDGNGENNYEE-141
HsCen2	
Loop III	113-FDDDETGKISFKNL-126
Loop IV	149-ADRDGDGEVSEQEF-162
Residues (red) of centrin which bind Ca ²⁺ .	

Table 1: CaM and Centrin EF-hand motifs reports the sequence of the four EF-hand motifs calmodulin and the two EF-hand motifs centrin showing the presence of acidic residues, which bind Ca²⁺.

Interaction of calmodulin and centrin with their cellular targets

CaM/centrin targets	CaM/Centrin binding motif of targets
Centrin	
Human XPC	COOH ⁸⁶³ -RKLRRERILLGKALLKWN-843 ^{NH2}
Human Sfi1R17	NH ₂ ⁶⁴¹ -RADLHHQHSLVLRALQAWV-660 ^{COOH}
Human Sac3	NH ₂ ¹²²⁵ -IFQTAKETLQELQCFCKYLQRWR1247 ^{COOH}
Transducin β	NH ₂ ³²³ -DMAVATGSWDSFLKIWN-340 ^{COOH}
CaM	
M=Murine Myosin V (IQ motif)	NH ₂ ⁷⁶⁹ -ACIRIQKTIRGWLLRKRY-786 ^{COOH}
Human MLCKII	NH ₂ ⁵⁶⁶ -KRRWKKNFIAVSAANRFKISS-587 ^{COOH}
Rat CaM-dependent Kinase II	NH ₂ ²⁹³ -FNARRKLGAILTTLAT-310 ^{COOH}
Residues (red) of target which bind centrin or CaM.	

Table 2: Binding motif of calmodulin targets, centrin targets reports the target-binding motifs for calmodulin and centrin.

Calmodulin targets

Calmodulin targets have been extensively reviewed [16] and include calmodulin-dependent kinase (CaMK), calmodulin-dependent phosphatase, death-associated protein kinase (DAPK), nitric oxide synthase (NOS), plasma-membrane Ca²⁺-ATPase (PMCA), calmodulin-activated adenylyl cyclase, and exotoxin secreted by *Bacillus anthracis*. The calmodulin-binding sites of calmodulin targets share several characteristics, including helical propensity, positive charge and hydrophobic residues. Calmodulin binds targets containing IQ-motifs (IQxxxRGxxxR) in binding mode 1-5-10 or 1-14. Rhoads and Friedberg [17] have studied the calmodulin-binding motifs of targets. Three recognition motifs exist for calmodulin interaction: one for Ca²⁺-independent binding and two for Ca²⁺-dependent binding (1-8-14 and 1-5-10 based on the position of conserved hydrophobic residues). The IQ motifs found in calmodulin targets have been reviewed [18]. Several examples of Ca²⁺-dependent or Ca²⁺-independent interactions of calmodulin with targets are listed below.

Calmodulin-CaMBD (small-conductance Ca²⁺-activated potassium channels (SK2))

To explore how target proteins may affect the affinity of calmodulin for Ca²⁺, the X-ray crystal structure of calmodulin in complex with the CaM-binding domain (CaMBD) from small-conductance Ca²⁺ activated potassium channels (SK2-b) has been determined at a resolution of 1.9 Å [19]. The splice variant SK2-b is less sensitive to Ca²⁺ than SK2-a. This study revealed that target proteins could change the conformation of calmodulin and regulate the affinity of calmodulin for Ca²⁺. Comparisons between SK2-a and SK2-b reveal differences in the C-lobe and in the linker of calmodulin bound to SK2. In addition, the four Ca²⁺-binding sites of calmodulin in complex with SK2-b are occupied, although the C-lobe of calmodulin in complex with SK2-a is free of Ca²⁺.

Calmodulin-Protein kinase

Calmodulin-dependent protein kinases catalyze the transfer of phosphate from the gamma position of ATP to the hydroxyl side chains of Ser and Thr residues of protein. Certain calmodulin-

dependent protein kinases are known to act on several substrates (e.g., CaMKI, CaMKII, and CaMKIV), whereas others act on specific substrates (e.g., CaMKIII, phosphorylase kinase, myosin light-chain kinases). Calmodulin-dependent protein kinase kinases have also been identified [20].

Calmodulin-Myosin Light-Chain kinase (MLCK)

Calmodulin-dependent smooth muscle myosin-light chain kinase is involved in the activation of smooth muscle contraction. The binding of calmodulin to MLCK creates a surface domain containing Lys30 and Gly40 and residues from the C-terminus of calmodulin, which leads to MLCK activation [21].

Calmodulin-Protein phosphatase (calcineurin)

Calcineurin is Ca²⁺-calmodulin-dependent Ser/Thr phosphatase. Calcineurin is a heterodimer (chain A and chain B); chain A contains the catalytic domain, and chain B consists of a regulatory domain that includes a calmodulin-binding region and an autoinhibitory domain. At low Ca²⁺ concentrations, calcineurin exists in inactive form in which the auto inhibitory domain is bound to the active site. Upon increases in Ca²⁺ concentrations, calmodulin binds Ca²⁺ and in turn binds to the regulatory domain of calcineurin, causing conformational changes that lead to the release of the auto inhibitory domain and the activation of the phosphatase [22].

Calmodulin-Myosin V

Myosins comprise a family of ATP-dependent motor proteins that are known for their role in muscle contraction [23]. Myosins (ATPases) move along actin filaments by coupling the hydrolysis of ATP to conformational changes. Cells contain various myosin isoforms. For example, myosin II (conventional myosin) generates muscle contractions, and myosin V (unconventional myosin) transports cargo in non-muscle cells. Myosin V consists of a globular motor domain that binds actin and hydrolyses ATP, which is connected to an elongated α-helical lever arm that contains six calmodulin-binding IQ motifs (IQxxxRGxxxR). Ca²⁺ activates the ATPase activity of full-length myosin V but not truncated myosin [24]. In the absence of

Ca²⁺, calmodulin binds myosin V, which adopts a folded inactive conformation. Low Ca²⁺ concentrations unfold and activate myosin, while higher Ca²⁺ concentrations dissociate calmodulin and inhibit myosin activity. The structure of apo-calmodulin bound to the first two IQ motifs of myosin V [25] has been solved and shows that the C-terminal lobe adopts a semi-open conformation when bound to the first part (IQxxxR) of the IQ motif, although the N-terminal lobe adopts a closed conformation when bound to the second part (GxxxR) of the IQ motif.

The CaM complexes structures, which are deposited in the Data Bank (PDB), have been reviewed [16]. The analysis revealed diverse binding modes and highlighted the conformational flexibility of CaM. Shukla et al. [26] constructed Markov state models from molecular dynamics simulations of the C-lobe of CaM in the Ca²⁺-bound and unbound regimes to explore contributions of a single domain of CaM to binding diversity.

Centrin targets

At least five proteins (XPC, Sfi1, Sac3, POC5, Transducin β) have been shown to bind centrin. Thus, centrin is involved in several cellular processes via its targets: DNA repair via XPC [27], spindle pole body duplication via Sfi1 [28], RNA export via Sac3 [29] and transduction via transducin β [30]. The hydrophobic pocket of centrin binds targets through a typical hydrophobic helix, W¹xxL⁴xxxL⁸. However, two sequence orientations of the centrin-binding motif exist, namely, W¹xxL⁴xxxL⁸ for XPC, and the reverse orientation, L8xxxL4xxW1, which is considered to be the amino acid sequence orientation for Sfi1 and transducin β . This is a unique characteristic compared with calmodulin.

Crystal [31-34] and NMR structures [35,36] of centrins in complex with truncated targets or target-derived peptides (i.e., XPC, Sfi1, or Sac3) have been solved. In all cases, the target-binding pocket is located in the C-terminal domain of centrin, and the F113 residue of human centrin is the main residue involved in target binding.

XPC

The structure from [36] shows that only residues in the C-terminal domain of human centrin 2 are involved in the interaction with the XPC peptide. Nine non-polar residues in human centrin 2 (L133, L112, M145, F113, M166, L126, A109, E105 and V129) form contacts with residues in the XPC peptide. The F113 and M145 residues form the pocket where the W residue of XPC binds.

Sac3

The structure of the CID region (proximal CTD-Interacting Domain) of Sac3 in complex with Sus1 and Cdc31 has been solved by X-ray crystallography [33,37]. In this complex, the W802 residue of Sac3 appears to play a central role in the interaction with the hydrophobic cavity of the centrin Cdc31. The F105 residue of Cdc31 in turn plays an important role in the association with Sac3.

Sfi1

A crystal structure of the centrin Cdc31 in complex with truncated Sfi1 has been solved by X-ray crystallography [34]. Both the C-terminal and N-terminal domains of Cdc31 form contacts with Sfi1. The structure also reveals several centrin-centrin interactions. These interactions suggest that a filament of several centrin molecules is

formed by centrin-centrin interactions and that Sfi1 stabilizes this filament through Sfi1 repeat-centrin interactions. An NMR structure of human centrin 2 in complex with the R17-Sfi1 peptide has also been solved [14]. Figure 3 shows the NMR structure of the C-terminal centrin 2 in complex with Sfi1.

Comparison of this structure with the NMR structure of the C-terminal domain of human centrin 2 in complex with P17-XPC peptide [35] led to the proposal that, the centrin 2 residue E148 discriminates between XPC and Sfi1 [14].

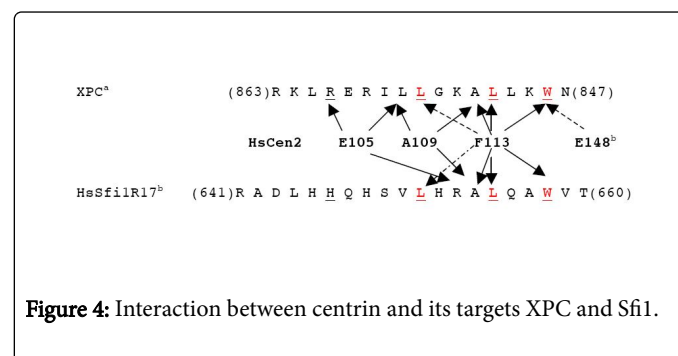
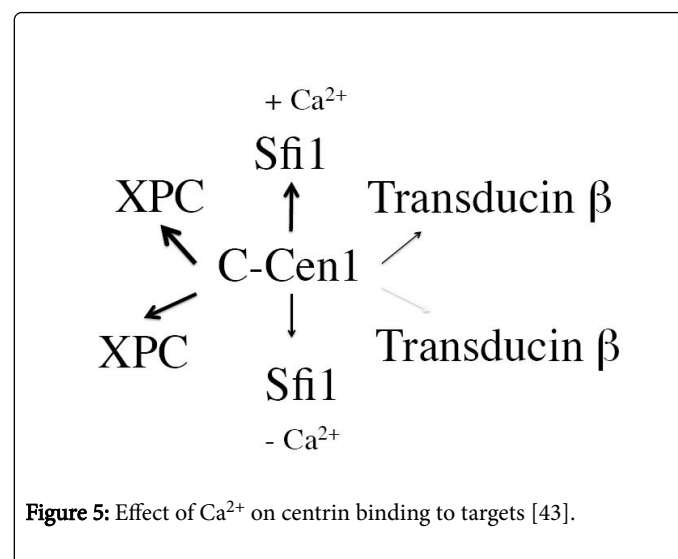


Figure 4 summarizes the residues (E105, A109, F113) of centrin, which bind the hydrophobic triad L8xxxL4xxW1 of Sfi1 or the reverse triad W1xxL4xxxL8 of XPC. As for CaM, Ca²⁺ binding to centrin can affect the binding of centrin to its targets. Thus, Figure 5 summarizes the effect of Ca²⁺ on centrin binding to its targets.



Other regulated processes: phosphorylation, ubiquitination, SUMOylation

Ca²⁺-dependent structural changes are modulated by other levels of regulation such as the posttranslational modification of proteins (e.g., phosphorylation, SUMOylation, ubiquitination).

Centrin is phosphorylated by MPS1 at residue T138, by CK2 at residue T138 and by Aurora-A at residue S170. The localization of centrin 2 to the centrosome is dependent on the availability of phosphorylatable T118 and functional calcium binding EF-hands [38]. Phosphorylation of human centrins 1 and 2 regulates the interactions

with Sfi1 and transducin β [39]. Figure 6 summarizes the effect of centrin phosphorylation on target binding.

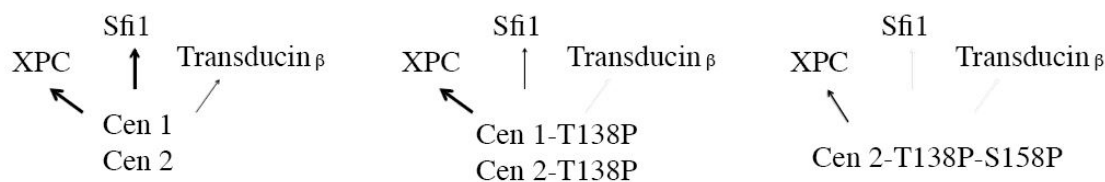


Figure 6: Effect of phosphorylation of centrin on binding to targets [43].

Centrins 1 and 2 are substrates of SUMO conjugation, and SUMOylation of centrin 2 is required for its efficient localization to the nucleus. XPC also undergoes SUMOylation, but the posttranslational modifications of XPC and centrin 2 are dependent on different factors [40]. SUMOylation of centrin 2 is also required for efficient interaction with XPC. The SUMOylation and ubiquitination of XPC drive DNA repair [41]. The Sfi1 C-terminal domain harbors phosphorylation sites for Cdk1 and the polo-like kinase Cdc5. Phosphoregulation of Sfi1 by Cdk1 has the dual function of promoting SPB separation for spindle formation and preventing premature SPB duplication [42]. The protein phosphatase Cdc14 has the converse role via phosphate group removal on Sfi1 [43].

Conclusion

Ca²⁺ regulates cellular functions via interactions between Ca²⁺-sensor proteins and targets that are involved in cellular processes. In addition, posttranslational modifications also regulate cellular processes. On some occasions, calmodulin, as well as centrin, bind to some of their targets in the absence of Ca²⁺. However, if a first step is Ca²⁺ independent, a second binding step can be Ca²⁺-dependent.

Acknowledgements

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Abbreviations Sfi1: Suppressor of fermentation-induced loss of stress resistance protein1; SPB: Spindle pole body; XPC: Xeroderma pigmentosum group C protein; Sac3: Is the central component of the transcription and mRNA export (TRESX-2) complex; CID: Proximal CTD-Interacting Domain; CaM: Calmodulin; MLCK: Myosin light-chain kinase.

References

- Carafoli E (2005) Calcium: a universal carrier of biological signals. Delivered on 3 July 2003 at the Special FEBS Meeting in Brussels. *FEBS J* 272: 1073-1089.

- Raggatt LJ, Partridge NC (2010) Cellular and molecular mechanisms of bone remodeling. *J Biol Chem* 285: 25103-25108.
- Swilius MT, Waxham MN (2008) Ca(2+)/calmodulin-dependent protein kinases. *Cell Mol Life Sci* 65: 2637-2657.
- Zeng H, Xu L, Singh A, wang H, Du L, et al. (2015) Involvement of calmodulin and calmodulin-like proteins in plant responses to abiotic stresses. *Frontiers in Plant Science* 6: 600
- Viridi AS, Singh S, Singh P (2015) Abiotic stress responses in plants: role of calmodulin-regulated proteins. *Frontiers in Plant Science* 6: 809
- Whalley H J, Sargent AW, Steele JFC, Lacoere T, Lamb R, et al. (2011) Transcription analysis reveals calcium regulation of specific promoter motifs in arabidopsis. *The Plant Cell* 23: 4079-4095
- Bhattacharya S, Bunick CG, Chazin WJ (2004) Target selectively in EF-hand calcium binding proteins. *Biochi Biophys Acta* 1742: 69-79.
- Friedberg F (2006) Centrin isoforms in mammals. Relation to calmodulin. *Mol Biol Rep* 33: 243-252.
- Gavet O, Alvarez C, Gaspar P, Bornens M (2003) Centrin4p, a novel mammalian centrin specifically expressed in ciliated cells. *Mol Biol Cell* 14: 1818-1834.
- Kretsinger RH, Nockolds CE (1973) Carp muscle calcium-binding protein .II structure determination and general description. *J. Biol. Chem.* 248: 3313-3326.
- Chattopadhyaya R, Meador WE, Means AR, Quiocho FA(1992) Calmodulin structure refined at 17 Å resolution. *J Mol Biol* 228: 1177-1192.
- Zhang M, Tanaka T, Ikura M (1995) Calcium-induced conformational transition revealed by the solution structure of apo calmodulin. *Nat Struct Biol*, 758-767.
- Kuboniwa H, Tjandra N, Grzesiek S, Ren H, Klee CB, et al. (1995) Solution structure of calcium-free calmodulin. *Nat Struct Biol* 2: 768-776.
- Martinez-Sanz J, Kateb F, Assairi L, Blouquit Y, Bodenhausen G, et al. (2010) Structure, dynamics and thermodynamics of the human centrin 2/hSfi1 complex. *J Mol Biol* 395: 191-204.
- Matei E, Miron S, Blouquit Y, Duchambon P, Durussel I, et al. (2003) C-terminal half of human centrin 2 behaves like a regulatory EF-hand domain. *Biochemistry* 42: 1439-1450.
- Tidow H, Nissen P (2013) Structural diversity of calmodulin binding to its target sites. *FEBS J* 280: 5551-5565.
- Rhoads AR, Friedberg F (1997) Sequence motifs for calmodulin recognition. *FASEB J* 11: 331-340.
- Bähler M, Rhoads A (2002) Calmodulin signaling via the IQ motif. *FEBS Lett* 513: 107-113.

19. Zhang M, Abrams C, Wang L, Gizzi A, He L, et al. (2012) Structural basis for calmodulin as a dynamic calcium sensor. *Structure* 20: 911-923.
20. Soderling TR (1999) The Ca-calmodulin-dependent protein kinase cascade. *Trends Biochem Sci* 24: 232-236.
21. Van Lierop JE, Wilson DP, Davis JP, Tikunova S, Sutherland C, (2002) Activation of smooth muscle myosin light chain kinase by calmodulin. *J Biol Chem* 277: 6550-6558.
22. Rumi-Masante J, Rusinga FI, Lester TE, Dunlap TB, Williams TD, et al. (2012) Structural basis for activation of calcineurin by calmodulin. *J Mol Biol* 415: 307-317.
23. Sellers JR (2000) Myosins: a diverse superfamily. *Biochim Biophys Acta-Molecular Cell Res* 1496(1): 3-22.
24. Kremontsov DN, Kremontsova EB, Trybus KM (2004) Myosin V: regulation by calcium, calmodulin, and the tail domain. *J Cell Biol* 164: 877-886.
25. Houdusse A, Gaucher JF, Kremontsova E, Mui S, Trybus KM, et al. (2006) Crystal structure of apo-calmodulin bound to the first two IQ motifs of myosin V reveals essential recognition features. *Proc Natl Acad Sci USA* 103: 19326-19331.
26. Shukla D, Peck A, Pande VS (2016) Conformational heterogeneity of the calmodulin binding interface. *Nature communications*.
27. Puumalainen M-R, R  thermann P, Min J-H, Naegeli H (2016) Xeroderma pigmentosum group C sensor: unprecedented recognition strategy and tight spatiotemporal regulation. *Cell Mol Sci* 73: 547-566.
28. Burns S, Avena JS, Unruh JR, Yu Z, Smith SE, et al. (2015) Structured illumination with particle averaging reveals novel roles for yeast centrosome components during duplication. *eLife*.
29. Fischer T, Rodriguez-Navarro S, Pereira G, Racz A, Schiebel E, et al. (2004) Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. *Nat Cell Biol*. 6: 840-848.
30. Trojan P, Raush S, Giessl A, Klemm C, Krause E, et al. (2008) Light-dependent CK2-mediated phosphorylation of centrins regulates complex formation with visual G-protein; *Biochem. Biophys. Acta* 1783: 1248-1260.
31. Thompson JR, Ryan ZC, Salisbury JL, Kumar R (2006) The structure of the human centrin 2-Xeroderma Pigmentosum Group C protein complex. *J Biol Chem* 281: 18746-18752.
32. Charbonnier JB, Renaud E, Miron S, Le Du MH, Blouquit Y, et al. (2007) Structural thermodynamic and cellular characterization of human centrin 2 interaction with Xeroderma Pigmentosum Group C protein. *J Mol Biol* 373: 1032-1046.
33. Jani D, Lutz S, Marshall NJ, Fisher T, K  hler A, (2009) Sus, Cdc, and the Sac3 CDI region form a conserved interaction platform that promotes nuclear pore association and mRNA export. *Mol Cell* 33: 727-737.
34. Li S, Sandercock A, Conduit P, Robinson CV, Williams RL, et al. (2006) Structural role of Sfi1p-centrin filaments in budding yeast spindle pole body duplication. *J Cell Biology* 173: 867-877.
35. Yang A, Miron S, Mouwad L, Duchambon P, Blouquit Y, et al. (2006) Flexibility and plasticity of human centrin 2 binding to the protein XPC from nuclear excision repair. *Biochemistry* 45: 3653-3663.
36. Thompson JR, Ryan ZC, Salisbury JL, Kumar R (2006) The structure of the human centrin 2-xeroderma pigmentosum group C protein complex. *J Biol Chem* 281: 18746-18752.
37. Dimitrova L, Valkov, Aibara S, Flemming D, McLaughlin SH, et al. (2015) Structural characterization of the chaetomium thermophilum TREX-2 complex and its interaction with the mRNA nuclear export factor Mex67:Mtr2. *Structure* 23: 1246-1257.
38. Dantas TJ, Daly OM, Conroy PC, Tomas M, Wang Y, et al. (2013) Calcium-binding capacity of centrin2 is required for Linear POC5 assembly but not for nucleotide excision repair. *PLoS One* 8: e68487.
39. Grecu D, Assairi L (2014) CK2 phosphorylation of human centrins 1 and 2 regulates their binding to the DNA repair protein XPC, the centrosomal protein Sfi1 and the phototransduction protein transducin β . *FEBS Open Bio* 4: 407-419.
40. Klein UR, Nigg EA (2009) SUMO-dependent regulation of centrin-2. *J Cell Sci* 122: 3312-3321.
41. van Cuijk, van Bell GJ, Turkyilmaz Y, Poulsen SL, Janssens RC, et al. (2015) SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair. *Nat Commun* 6: 7499.
42. Elserafy M, Saric M, Neuner A, Lin T-C, Zhang W, et al. (2014) Molecular mechanisms that restrict yeast centrosome duplication to one event per cell cycle. *Current Biol* 24: 1456-1466.
43. Avena JS, Burns S, Yu Z, Ebmeier CC, Old WM, et al. (2014) Licensing of yeast centrosome duplication requires phosphoregulation of Sfi1. *Plos Genetics* 10: e1004666.