

Centrin Phosphorylation and Localization Dynamics during the Centriole Cycle

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Centrin was first discovered as a major component of basal body-associated contractile flagellar roots in the algae, and subsequently was found to be a common protein of centrosomes [1-3]. Four centrin genes are expressed in mammals: *Cetn-1* through *Cetn-4* [4-7]. Centrin-2 and -3 are centriole proteins and recombinant GFP-centrin-2 and centrin-3 localize to centrioles and also to the pericentriolar material that surrounds the centrioles [2, 3, 8, 9].

Centrin is highly conserved in sequence and shows several striking structural features outside the four calcium-binding EF-hand domains. The carboxy-terminal half of centrin is highly conserved, and the short carboxy-terminal sequence -₁₆₇KKTS_{LY172}- is conserved among centrins associated with centrioles and basal bodies. This sequence is missing in human centrin-3 and in centrins of lower eukaryotes lacking centrioles or in centrins that localize to cellular structures other than centrioles. Three putative Mps1 phosphorylation sites that augment the canonical centriole assembly pathway have been identified on centrin-2 [10]. Earlier, phosphorylation of centrin-2 at a distinct carboxy-terminal phosphorylation site (-KKTS^{170P}LY) by protein kinase A was shown to promote centriole separation [11]. Finally, abnormal centrin phosphorylation S170 is observed in human breast tumors that have aberrant centriole numbers [12], suggesting that phosphorylation of centrin must be tightly regulated.

Here, we will present observations that show that in a mitogen-synchronized cell cycle, centrin abundance increases progressively and becomes phosphorylated at residue S170 prior to centriole duplication and DNA synthesis. Phospho-S170-centrin is asymmetrically localized to one of the pre-existing centrioles at about the time that primary cilia are lost. The appearance of phospho-S170-centrin is temporally coincident with recruitment of robust SAS-6 staining to both pre-existing centrioles. A second peak of centrin S170 phosphorylation is seen at the onset of mitosis, preceding the late-mitotic ablation of centrin protein. Cells expressing conditional mutations at S170 (S>A₁₇₀ and S>D₁₇₀) in centrin-2 show altered frequency of primary cilia and a unique pattern of PCM-satellite material reminiscent of failed centriole assembly.

The timing and localization of centrin phosphorylation and the opposing effects of conditional centrin mutations engineered to either block or mimic centrin phosphorylation suggest the intriguing possibility that centrin harbors a carboxy-terminal phosphorylation-sensitive alteration that corresponds to key events in the centriole cycle.

References

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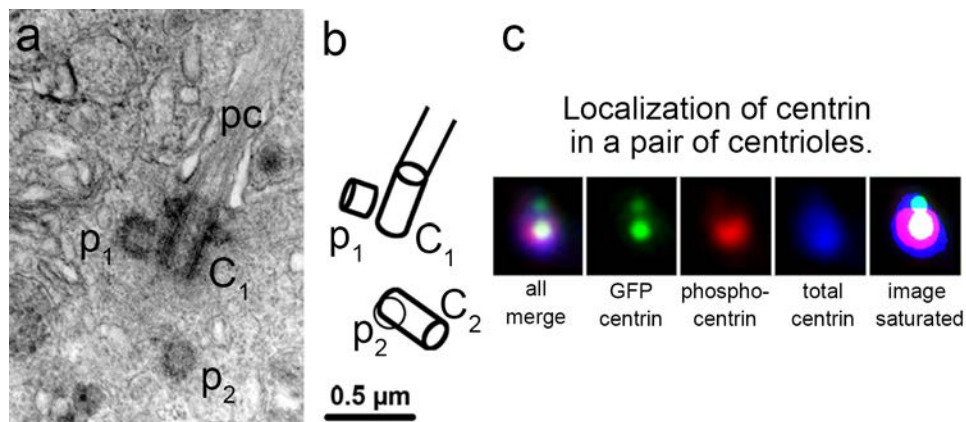


Figure Legend. a) Electron micrograph through a centrosome showing a primary cilium (pc), its centriole (C₁), and two procentrioles (p₁ and p₂). b) Diagram illustrating the essential features shown in (a) with additional information from adjacent serial sections. c) Fluorescence microscopic localization of centrin in a pair of centrioles, showing a merged image of all channels, GFP-centrin2, phosphor-centrin at the carboxy-terminal sequence KKTS^PLY, total centrin, and a model image with each of the channels adjusted to saturation.