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Plk4/SAK/ZYG-1 in the regulation of centriole duplication

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Abstract

Centrioles organize both centrosomes and cilia. Centriole duplication is tightly regulated and coordinated with the cell cycle to limit duplication to only once per cell cycle. Defects in centriole number and structure are commonly found in cancer. Plk4/SAK and the functionally related *Caenorhabditis elegans* ZYG-1 kinases initiate centriole duplication. Several recent studies have elucidated the regulated activity of these kinases and potential downstream targets for centriole assembly.

Introduction and context

Centriole assembly facilitates the formation of two centrosomes with a pair of resident centrioles at their core, providing the basis for bipolar spindle organization during mitosis. Duplication also ensures that each daughter cell receives a pair of centrioles with one old, mother centriole and one new, daughter centriole [1,2].

Centriole biogenesis occurs via a series of conserved morphological stages that culminate in a cylindrical structure that is competent to recruit pericentriolar material for centrosome function and to nucleate cilia (reviewed in [3-7]). The new daughter centriole is first detected as an amorphous electron density and a cartwheel adjacent to the proximal end of the mother centriole. The cartwheel is composed of a central tubule of approximately 25 nm in diameter and nine spokes that extend outward from the central tubule. Triplet microtubules form at the end of each spoke to form the pro-centriole. The pro-centriole then matures into a centriole by additional triplet microtubule growth and the assembly of accessory molecular components and structures.

The coiled-coil protein Sas6 is a conserved component of central tubules that is essential for central tubule and, ultimately, centriole formation [8-14]. In *Drosophila*, overexpression of Sas6 initiates both central tubule and centriole formation, suggesting that this protein is important for early centriole assembly, and possibly limiting in the process [13,15]. Furthermore, the centriole functions of the cartwheel protein Bld10 [10], and of SAS-4/CPAP (centrosomal P4.1-associated protein), which directs microtubule assembly [16-19], both depend on prior Sas6 function in the pathway. It is this core group of proteins (Sas6, Sas4 and Bld10) that make up a conserved, ancestral module [20,21]. Cartwheels are thought to be dynamic in that they are required to initiate pro-centriole assembly but, in many cell types, are no longer detected in mature centrioles [17,22]. Consistent with the central tubule loss from mature centrioles, Sas6 also disappears with centriole maturation in human cells whereas Sas6 and the central tubule/cartwheel remain at centrioles in *Chlamydomonas* and *Tetrahymena*, in which the cartwheel is detected in mature centrioles [10,11,22].

A number of models exist for the licensing and regulation of new centriole assembly (reviewed in [1,2,23]). One conserved mechanism is the regulation of centriole assembly by the Polo-like kinase Plk4/SAK, and the functionally related kinase *Caenorhabditis elegans* ZYG-1 [20,21,24-26]. Two recent studies have explored the evolution of these protein kinases [20,21]. Here, we

review additional studies that begin to uncover the regulation of Plk4/SAK/ZYG-1 and how these kinases may directly activate Sas6.

Major recent advances

Regulating Plk4/SAK/ZYG-1 kinase activity

Plk4/SAK/ZYG-1 localizes to centrioles and centrosomes and is required for normal duplication [24,25,27,28]. Active Plk4/SAK kinase is present at duplicating mother centrioles during G1/S and the protein levels increase at both centrioles into mitosis [29]. In addition to centriole localization, Plk4/SAK protein levels are regulated and, when aberrant, centriole assembly is either amplified or decreased corresponding to levels of Plk4/SAK [30,31]. Defects resulting in either too much or too little Plk4/SAK are deleterious and correlate with chromosome instability (CIN) and cancer [32,33].

Plk4/SAK is a low abundance protein that is SCF/Slimb ubiquitinated and targeted for destruction by the 26S proteosome in *Drosophila* [34-36]. This destruction limits centrosome/centriole amplification. Importantly, the SCF-mediated destruction of Plk4/SAK is also affected by its autophosphorylation at a phosphodegron motif [37]. Thus, Plk4/SAK self-regulates its activity by phosphorylation to promote its own destruction. In addition to protein destruction, Plk4/SAK phosphorylated in the phosphodegron (S305) is differentially localized to the centrosome pericentriolar material [29]. This may be a mechanism of sequestration to further control the activation of centriole duplication. As Plk4/SAK kinase activity increases, the protein becomes destroyed and re-localized in a self-regulating mechanism to limit centriole re-duplication. Plk4/SAK levels become highest during mitosis. Finally, centrosome levels of *C. elegans* ZYG-1 are also regulated by the conserved, putative RNA binding protein SZY-20 [38]. Thus, the regulation of Plk4/SAK/ZYG-1 appears to undergo multiple mechanisms of regulated activity, reflecting the tight cell cycle coordination and potentially multiple cellular functions for these kinases.

Capitalizing on the multiple mechanisms for regulating Plk4/SAK/ZYG-1 kinases, variant activity is observed depending on cell type and cell cycle differences. For example, *C. elegans* ZYG-1 is differentially regulated during mitosis and meiosis via dissimilar localization dependencies and this may reflect both positive and negative mechanisms for ZYG-1 activity in centriole duplication [39].

What are the Plk4/SAK/ZYG-1 kinase substrates?

The target(s) of Plk4/SAK/ZYG-1 phosphorylation for centriole assembly is not well understood. However, ZYG-1 was recently found to phosphorylate SAS-6 at Ser123 in *C. elegans* [40]. Ser123 is not a conserved residue in the Sas6 protein family, suggesting that the site of regulation is divergent, as are the kinases controlling centriole duplication. Consistent with conservation in the regulatory mechanism, however, HsSas6 localization is, in part, regulated by Plk4 [22].

SAS-6 mutations that mimic the phosphorylation (S123D) can restore centriole duplication in a partial ZYG-1 knockdown [40] that would normally arrest cells with monopolar spindles due to inhibition of centriole duplication [28]. Despite its ability to compensate for a partial loss of ZYG-1 function, the phospho-mimetic SAS-6 mutant cannot rescue the complete loss of ZYG-1, indicating that ZYG-1 has other roles in promoting centriole duplication.

SAS-6 S123 phosphorylation is required for normal SAS-6 maintenance at centrioles [40]. The protein localization dynamics suggest a model in which SAS-6 phosphorylation is not required for recruitment to centrioles but S123 phosphorylation is required to maintain SAS-6 at centrioles later in the cell cycle. This is consistent with human studies where Plk4 is required to maintain Sas6 at centrioles during mitosis and less so during interphase [22].

Future directions

The coordination of centriole assembly with the cell cycle is critical for regulated spindle assembly. A series of recent studies describing the regulation and a downstream target of Plk4/SAK/ZYG-1 protein kinases has begun to describe the complexity of this process.

Perhaps the most intriguing feature of these studies is the observation that Plk4/SAK activity peaks at mitosis and promotes maintenance of Sas6 during this same time in the cell cycle. While both of these proteins are clearly important to initiate centriole assembly, multiple functions are revealed by these studies. An added layer of complexity also comes from the differences between ZYG-1 regulation in mitotic and meiotic cells [39]. In addition to its role in centriole function, Plk4's cell division role is likely to be more complex, with key functions in cytokinesis. Plk4 may regulate Rho GTPase and cytokinesis by phosphorylating the Rho guanine nucleotide exchange factor Ect2 [41]. Altered Plk4 activity may also drive carcinogenesis by disrupting Plk4's cytokinesis function [41,42].

Finally, the phosphorylated and unphosphorylated forms of Sas6 may have unique properties to facilitate both central tubule assembly and other functions that remain

to be discovered. New central tubule formation for the initiating stages appears to not require Sas6 phosphorylation. Along these lines, such modification may negatively regulate the Sas6 oligomerization that can generate tubule formation *in vitro* [43]. The differential phospho-Sas6 may be required for centriole maturation to form a stable structure. Beyond its regulation are questions concerning how Sas6 contributes to central tubule formation and the creation of ninefold radial symmetry in attachment of the cartwheel spokes to the central tubule.

Abbreviations

Plk4, polo-like kinase 4; SAK, Snk/Plk-akin kinase; Sas6, spindle assembly abnormal protein 6 homolog; SCF, Skp, Cullin, F-box containing complex.

Competing interests

The authors declare that they have no competing interests.

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