Centrosomes as Scaffolds: The Role of Pericentrin and Protein Kinase A-anchoring Proteins

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Abstract

The centrosome, consisting of the centrioles and pericentriolar material, is a dynamic structure that organizes several proteins involved in cell cycle regulation. Pericentrin and protein kinase A-anchoring proteins (AKAPs) are proteins that associate into complexes at the centrosome. Both bind protein kinase-A (PKA), bringing PKA to the centrosomal subcellular compartment where it serves a regulatory function by interacting with proteins such as Op18/stathmin, which is involved in microtubule dynamics. Pericentrin also binds the microtubule nucleating γ-tubulin ring complex (γ-TuRC). This review presents recent findings that pericentrin and AKAPs act as scaffold proteins that recruit regulatory molecules to the centrosome.

Introduction

E.B. Wilson (1928) attributes the earliest discovery of the centrosome to Theodor Boveri who, in 1901, described it as a central body containing “centroplasm” and the centriole. Wilson continues, “in its simplest form the central body appears under the highest powers as a single granule of extreme minuteness…at the focus of astral rays” (Wilson, 1928). Since 1928, the role of the centrosome has continued to be refined. It is now referred to as the cell’s MTOC, a dynamic structure that assumes different forms and locations depending on the cell cycle phase (Brinkley, 1985). During the G1 phase of the cell cycle the centrosome, or MTOC, consists of a single pair of organelles called centrioles. Each centriole is constructed from nine triplet microtubules and forms at a right angle to its partner centriole. At the G1/S boundary, when DNA replication commences, the centrioles begin to separate slightly. At this time, each centriole will duplicate semiconservatively, and the new centriole will elongate during the G2 phase, again orthogonal to its partner centriole. Two centriole pairs are now contained within one centrosome. At the initiation of the mitotic phase, the centriole pairs move to opposite sides of the nuclear envelope and recruit half of the pericentriolar material (PCM) with them (Kellogg et al., 1994). As the nuclear envelope breaks down at the end of prophase, the microtubules originating from the centrosomes begin to polymerize and depolymerize in a process called dynamic instability (Mitchison and Kirschner, 1984a, 1984b). As the tubulin dimer pool decreases during polymerization, the probability of depolymerization increases. Once depolymerization is initiated, the microtubule is completely taken apart, increasing the dimer pool and leaving a free nucleation site (Mitchison and Kirschner, 1984a). Rates of polymerization and depolymerization depend on the state of the tubulin dimer (GTP versus GDP) at the plus end of the microtubule (Mitchison and Kirschner, 1984b). Dynamic instability continues until kinetochores attach to the mitotic spindle and align chromosomes on the metaphase plate. The kinetochore is a specialized protein complex that assembles on a discrete region of the chromosome called the centromere. Following chromosome alignment, the cell proceeds into anaphase (Merdes and Cleveland, 1997).

This review focuses on the role of the centrosome in the cell cycle and how specific proteins such as pericentrin and protein kinase A-anchoring proteins (AKAPs) may facilitate centrosome activity by serving as structural and regulatory scaffolds for those proteins that regulate cell cycle events (Figure 1). Pericentrin’s putative role as a scaffold protein comes from structural analysis as well as from studies that elucidate its interaction with complexes and signaling molecules at the centrosome (Doxsey et al., 1994; Merdes and Cleveland, 1997; Dictenberg et al., 1998). Pericentrin’s association with γ-tubulin and protein kinase A (PKA) makes it an interesting scaffold for regulation of cell cycle events and a possible target for cancer therapy.

The Centrosome

The centrosome appears as bodies of electron-dense material either diffused throughout the cytoplasm or specifically localized around centrioles. The centrosome serves as the cell’s MTOC, a dynamic structure that assumes different forms and locations depending on the cell cycle phase (Brinkley, 1985). As the nuclear envelope breaks down at the end of prophase, the microtubules originating from the centrosomes begin to polymerize and depolymerize in a process called dynamic instability (Mitchison and Kirschner, 1984a, 1984b). As the tubulin dimer pool decreases during polymerization, the probability of depolymerization increases. Once depolymerization is initiated, the microtubule is completely taken apart, increasing the dimer pool and leaving a free nucleation site (Mitchison and Kirschner, 1984a). Rates of polymerization and depolymerization depend on the state of the tubulin dimer (GTP versus GDP) at the plus end of the microtubule (Mitchison and Kirschner, 1984b). Dynamic instability continues until kinetochores attach to the mitotic spindle and align chromosomes on the metaphase plate. The kinetochore is a specialized protein complex that assembles on a discrete region of the chromosome called the centromere. Following chromosome alignment, the cell proceeds into anaphase (Merdes and Cleveland, 1997).

In 1977, Gould and Borisy showed that the intact centrosome, consisting of centrioles surrounded by PCM, could be isolated from any cell cycle phase and could function as an organelle in itself. They observed the attachment of microtubule arrays to centrosomes in both dividing and interphase cells. Two observations suggested that the anchoring of microtubules occurred at the PCM and
not at the centriole: 1) After recovery from a colcemid block, native microtubules attached to the PCM and not to the centrioles and 2) When PCM was removed, the centrioles alone did not produce microtubule asters when incubated with purified tubulin. Numerous proteins have now been found to associate with the centrosome. These include kinases, phosphatases, the p53 tumor suppressor protein, and the γ-tubulin structural protein (Brinkley and Goepfert, 1998).

**Pericentrin - A Structural Scaffold**

Using antisera from scleroderma patients, Tuffanelli et al. (1983) identified a 220 kDa protein that by immunofluorescence localized to the centrosome. This protein, named pericentrin, was found to form an integral part of centrosomes and non-centriolar MTOCs (Doxsey et al., 1994). The amino acid sequence of pericentrin predicts a tripartite structure with a central alpha-helical domain flanked by C- and N-terminal non-helical domains (Doxsey et al., 1994). This structure is similar to that of intermediate filaments, suggesting that pericentrin may dimerize in a coiled-coil leaving the C- and N-termini to interact with associated proteins (Albers and Fuchs, 1992). The flanking domains contain a number of protein kinase recognition sequences (Doxsey et al., 1994). Using intermediate filament dimerization and filament formation patterns of the nuclear lamins as a model (Aebi et al., 1986), pericentrin can be envisioned to wrap multiple times around the centrioles. Such a structure could provide docking for multiple proteins at the centrosome (Doxsey et al., 1994). By enhanced immunofluorescence and image deconvolution, pericentrin’s three-dimensional structure appears as a reticular lattice structure which increases in complexity and size as the cell cycle progresses from G1 to mitosis (Dichtenberg et al., 1998).

Structurally, pericentrin could efficiently tether multiple proteins and form a complex that could serve as a scaffold. When pericentrin is disrupted by injection of anti-pericentrin antibodies in mouse oocytes, the microtubule organization is also disrupted (Doxsey et al., 1994). Microtubules now form arrays with loose foci near the cell cortex. These results indicate that pericentrin influences aster formation in immature centrosomes and is perhaps one of the requirements for microtubule nucleation in vivo.

**Pericentrin and γ-Tubulin - A Microtubule Nucleating Complex**

γ-Tubulin is an essential component of the centrosome and links the centrosome to microtubules (Sears and Kirschner, 1994). γ-Tubulin was localized to ring structures in the PCM of purified centrosomes by immunogold electron microscopy (Moritz et al., 1995). Zheng et al. (1995) showed that this γ-tubulin ring complex (γ-TuRC) could nucleate microtubules in vitro through binding at microtubule minus ends. The orientation of the microtubule with respect to the γ-TuRC was determined by analyzing differences in the growth rates of microtubules at their plus- and minus-ends. Given that the plus end of the microtubule grows approximately three times faster than the minus end in the presence of free tubulin, the rates of the two ends were compared after addition of γ-TuRC by pulsing varying concentrations of rhodamine-labeled tubulin. From these results, a seed-nucleation model was proposed where the γ-TuRC stabilizes the minus end of the growing microtubule at the centrosome (Zheng et al., 1995).

Using Xenopus eggs as a source of centrosome components, Dichtenberg et al. (1998) employed several biochemical methods to demonstrate the interaction of pericentrin with γ-tubulin in a complex. Both pericentrin and γ-tubulin were detected in high density sucrose gradient fractions. However, in vitro-transcribed pericentrin migrated in the low density fraction. Pericentrin and γ-tubulin also co-eluted by gel filtration, indicating that they are either part of the same complex or of different complexes with similar biochemical properties. Immunoprecipitation assays using Xenopus and mammalian cellular extracts confirmed formation of complexes between γ-tubulin and pericentrin. Nucleated microtubules associated with the lattice in a predicted ratio of one pericentrin complex to two γ-tubulin complexes. From these data, Dichtenberg et al. (1998) postulated that pericentrin may be necessary for γ-tubulin complexes to assemble at the centrosome.

Pericentrin associates with γ-tubulin in complexes at the centrosome and also influences microtubule nucleation patterns. This suggests that either the correct binding of γ-TuRC to pericentrin regulates the ordering of the microtubule arrays or that other proteins that also bind pericentrin help regulate γ-TuRC nucleating activity.

**AKAPs - Regulatory Scaffolds**

Also, the recently described centrosomal AKAPs serve a scaffolding role at the centrosome (Witczak et al., 1999; Schmidt et al., 1999). AKAPs act as scaffolds to recruit PKA to subcellular compartments. PKA, which is made up of regulatory (R) and catalytic (C) subunits (R2C2 in the holoenzyme), is regulated by cyclic AMP (Beebe and Corbin, 1986). Like pericentrin, the centrosomal AKAPs have coiled-coil domains that are characteristic of structural proteins (Witczak et al., 1999). In 1999, both Witczak et al. and Schmidt et al. described novel AKAPs that localize to the centrosome and tether PKA by binding to the regulatory subunit (R). AKAP450 was identified by RI overlay screening of a Jurkat T lymphocyte expression library using 32P labeled RII as a probe and also by using antibodies to rabbit AKAP120 (Witczak et al., 1999). AKAP350 was also identified using rabbit AKAP120 antibody and is involved in both centrosomal
and noncentrosomal regulation of cytoskeletal systems by anchoring PKA (Schmidt et al., 1999). During the cell cycle, AKAP350 was localized to the centrosome. It was also found at the cleavage furrow in anaphase and telophase cells, provided a possible role for AKAP350 in the contractile ring that forms during telophase (Witczak et al., 1999). The localization of AKAPs and their ability to recruit PKA to subcellular compartments implies a scaffold-type regulation of PKA during many phases of the cell cycle. Specific PKA signaling is postulated to result at least in part from its proximity to substrate, and prevented cross-talk with other PKA-signaling pathways (Pawson and Scott, 1997).

PKA has been shown to affect microtubule organization (Melander-Gradin et al., 1998; Lane and Kalderon, 1994). Mutations in PKA caused mislocalization of RNAs by disrupting microtubules in Drosophila (Lane and Kalderon, 1994). PKA has also been associated with microtubule dynamics by its interaction with Op18/stathmin. Op18 interacts preferentially with unpolymerized microtubule subunits, increasing the catastrophe (depolymerization) rate and regulating microtubule dynamics in response to external signals (Belmont and Mitchison, 1996). Melander-Gradin et al. (1998) investigated the role of PKA in phosphorylation of Op18 using ectopic expression of the c-myc-tagged catalytic subunit of PKA and Flag-tagged Op18. Op18 phosphoisomers were detected with anti-Op18 antibodies using native PAGE (PolyAcrylamide Gel Electrophoresis) to separate Op18 according to differences in charge caused by phosphorylation at each of four identified phosphorylation sites. Two of these sites (Ser-16 and Ser-63) are phosphorylated by PKA. Phosphorylation of these two serines was shown to downregulate Op18 microtubule destabilizing activity. This was determined by measuring cellular microtubule polymer content in Op18 and PKA co-transfected cells (Melander-Gradin et al., 1998). In summary, AKAPs are present in regions of high microtubule nucleating activity. PKA is recruited to the AKAPs where it can influence microtubule dynamics by regulating microtubule-stabilizing factors.

Linking Structural and Regulatory Scaffolds

Diviani et al. (2000) elucidated a link between pericentrin and PKA. They showed that pericentrin alone can anchor PKA by binding RII and tethering it at the centrosome, therefore, giving pericentrin a role similar to AKAPs. Pericentrin, RII, and the catalytic subunit of PKA were all detected in pericentrin immunoprecipitates (Diviani et al., 2000). These results indicate that, similar to AKAPs, pericentrin acts to concentrate PKA at specific subcellular compartments.

Pericentrin and Cancer

Genetic instability, resulting from improper regulation at the centrosome, can lead to tumor formation. Theodor Boveri first proposed a role for centrosomes in cancer in 1914 and suggested that cancer cells could arise due to improper activity or replication of centrosomes. The result would be a gain or loss of chromosomes during mitosis (Salisbury et al., 1999; Brinkley and Goepfert, 1998). Centrosome abnormalities can affect the cell in multiple ways. They can lead to improper centrosome segregation and affect cell polarity. Vesicular trafficking
can also become disoriented when multiple MTOCs are present within the cell. Aberrant protein scaffolding due to centrosomal abnormalities can lead to improper or a complete lack of centrosome localization of cell cycle-dependent kinases, such as PKA, Cdc2, and check-points such as p53 (Salisbury et al., 1999).

Abnormal pericentrin levels have been observed in tumor cells. Pihan et al. (1998) used immunoperoxidase staining with an antipericentrin antibody in tissues and in cell lines from breast, lung, prostate, colon, and brain cancers. Normal cells in interphase labeled a single dot with anti-pericentrin antibodies. In mitotic cells, a pair of dots were observed, one at each pole. Tumor tissue had a much higher level of pericentrin expression, forming multiple foci that are sometimes interconnected by atypical pericentrin filaments. This diffuse, patchy pericentrin label often localized to structures that contained no centrioles. Yet, all pericentrin-labeled structures were able to nucleate the growth of new microtubule asters regardless of the size, number, or shape of the pericentrin foci (Pihan et al., 1998). Overexpression or mislocalization of pericentrin could sequester regulatory proteins away from functional centrosomes, and this situation could lead to defects in the mitotic spindle and chromosome segregation, which is a hallmark of tumor cells.

Conclusions

Due to improved deconvolution algorithms, and immunofluorescence techniques, several interesting proteins have been discovered at the PCM. Of these, pericentrin and the AKAPs are implicated in scaffolding roles at the centrosome (Witczak et al., 1999; Schmidt et al., 1999; Doxsey et al., 1994; Merdes and Cleveland, 1997). The nature of these proteins allows one to envision the PCM as a complex scaffold designed to coordinate proteins for proper and efficient function. Pericentrin may serve as a structural scaffold necessary for microtubule nucleation in cooperation with γTuRCs. It may also act as a link between structural elements like the microtubule network and regulatory elements like PKA and downstream targets. Determining the function of the centrosomal scaffold proteins, pericentrin, and the AKAPs, as well as the proteins and signaling molecules they bring together, may have important implications for understanding tumorigenesis.

References


