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Regulation and Regulatory Activities of Centrosomes

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Abstract The centrosome functions in the organization of the cytoskeleton, in specification of cell polarity, and in the assembly of the bipolar spindle during mitosis. These activities are largely the result of microtubule nucleation activity and the centrosome's structural influence on the form of the microtubule array that it anchors. Centrosome duplication and microtubule nucleation activity are precisely regulated during development and the cell cycle. Loss of normal centrosome regulation and function may lead to alterations in cell polarity and to chromosomal instability through mitotic defects resulting in aneuploidy. This is particularly true for many malignant tumors. Here, we review the regulation and regulatory activities of centrosomes and consider some of the questions of current interest in this area. J. Cell. Biochem. Suppls. 32/33:192–199, 1999. © 1999 Wiley-Liss, Inc.

Key words: breast cancer; centriole; kinase; phosphatase; mitosis

The cytoskeleton plays an essential role in organizing the cytoplasm during interphase, and the mitotic spindle throughout mitosis. The centrosome is the major microtubule-organizing center (MTOC) of the cell. The centrosome contains two orthogonally positioned barrel-shaped centrioles composed of nine, radially positioned, triplet microtubule bundles. The older of the two centrioles, the mother centriole, can be distinguished from the younger daughter centriole by the presence of projections at its distal end. The proximal ends of the two centrioles are connected by fibrous material that displays Ca^{2+} sensitivity [Paintrand et al., 1992]. The centriole pair is surrounded by a mass of amorphous pericentriolar material (PCM) that contains a focus of both structural and enzymatic proteins. These proteins function in the initiation and regulation of microtubule nucleation.

One unique feature of the centrosome is its ability to duplicate once, and only once, during each cell cycle. This duplication process begins at the G_1/S transition and is initiated when the centrioles separate from each other and, in what may be a template driven process, a new daughter centriole is formed in an orthogonal position to its parent. Each new centriole pair recruits half of the PCM and they migrate to opposite sides of the cell during mitosis. The presence of only two centrosomes in the cell as it enters mitosis ensures the formation of two spindle poles and the equal segregation of sister chromatids to each daughter cell. Disruption of centrosome duplication can lead to multipolar or monopolar mitosis, the unequal segregation of chromosomes and aneuploidy.

Aneuploidy and genomic instability are common features of solid tumors. This raises the possibility that abnormal centrosome function may lead to the development of aneuploidy and the progression of some cancers. Renewed emphasis on centrosome research has lead to the discovery of molecular components that appear to be directly involved in regulating centrosome function. The focus of this review is on (1) recent advances in the identification and functional analysis of novel centrosomal components, and (2) how correct centrosome function regulates proper cytoskeletal organization to ensure normal cell function.

CENTROSOME REGULATION

Kinases and Their Associated Proteins

The activity of protein kinases and phosphatases control many cellular processes. The phosphorylation/dephosphorylation of proteins can regulate their function, localization and half-life. Many kinases have been found to localize at the centrosome, these include $p34^{cdc2}$, Plk1, aurora 2/ STK15 (BTAK), Aik, cAMP-depen-

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dent kinase II (PKA), fyn, Ca^{2+}/calmodulin-dependent kinase, and Nek2 [for review, see Brinkley and Goepfert, 1998]. However, the presence of a protein at the centrosome does not necessarily indicate an essential centrosomal function. Some proteins present at the centrosome may merely be passengers that reside there during the cell cycle to ensure that following cytokinesis both daughter cells receive at least some portion of the protein. This is the case for certain chromosomal proteins [Earnshaw and Bernat, 1991; Rattner et al., 1992]. While many kinases have been localized to the centrosome, what remains to be discovered for most of them are their relevant centrosome associated substrates and their function.

Centrosomes begin their semiconservative duplication at the G_1/S transition and continue their development until they separate at G_2/M. Nek2 kinase activity correlates with the period of centrosome duplication. Nek2 is the human member of the NIMA related kinases that exhibit specific mitotic effects [Fry et al., 1998b]. Nek2 associates with the centrosomes throughout the cell cycle in a manner independent of microtubules. Overexpression of Nek2 results in the unique phenotype of inducing inappropriate centriole splitting, disassociation, and eventual disintegration. It is believed that centriole splitting is an early step in duplication, indicating that Nek2 may be involved in the initiation of this process. Nek2 also has a potential centrosomal substrate that has been identified, C Nap1(Cep250) [Fry et al., 1998a; Mack et al., 1998]. C-Nap1 is a large protein localized to the proximal end of centrioles in a microtubule-independent manner during interphase, but not during mitosis. It has been proposed that the phosphorylation of C-Nap1 by Nek2 results in the initial separation of centrosomes.

What targets a particular protein to the centrosome? A consensus localization sequence common to proteins that reside at the centrosome has not been identified. How does a kinase, that is not exclusively centrosomal, become localized to the centrosome at the correct time during the cell cycle? Protein kinase A (PKA) is an example of this phenomenon. It is found in many regions of the cell, including the centrosome, but until recently, what specifically targeted a portion of it to reside at the centrosome was unknown. The identification of two centrosomal protein kinase A anchoring proteins, AKAP350 and AKAP450 (CG-NAP) that directly interact with the type IIβ regulatory subunit of PKA, have helped solve this question [Schmidt et al., 1999; Takahashi et al., 1999; Witczak et al., 1999]. The AKAPs have the ability to bind to the regulatory subunit RIIβ of PKA and thus ensure that PKA is present in a position that facilitates a close association with its centrosomal substrates. The AKAPs may provide a structural base for these interactions by virtue of their large regions of coiled-coil tertiary structure, a common motif of many structural centrosomal proteins. AKAP350 also has a 20% similarity to C-Nap1 (CEP250), indicating that these two proteins may possess some degree of functional overlap [Schmidt et al., 1999]. AKAP 450 has also been shown to bind to and interact with the catalytic subunit of protein phosphatase 2A and 1 [Takahashi et al., 1999]. This is unique in that one structural centrosomal protein has the ability to bind to both a kinase and phosphatase that both localize to the centrosome. It will be of interest to determine if additional kinases are localized through the direct action of specific centrosome-targeting proteins.

Phosphatases

Similarly, phosphatases have recently been identified to localize at the centrosome. The involvement of protein phosphatase 2A in regulating proper *Drosophila* centrosome function was recently demonstrated [Snaith et al., 1996]. Protein phosphatase 2A may play a role in coupling the nuclear and centrosome cycles. Protein phosphatase 4 (PP4) has also been localized to centrosomes in both *Drosophila* and mammals. Genetic manipulation implicates PP4 action in microtubule nucleation and stabilization [Helps et al., 1998]. Protein phosphatase 1 has three isoforms α, γ, and δ [Andreassen et al., 1998]. During interphase, the α isoform is largely found associated with the nuclear matrix but, during mitosis, becomes localized to the centrosome. This pattern of localization is seen for many other centrosomal proteins including NuMA. The identification of different phosphatases that localize to the centrosome supports the concept that multiple regulatory phosphorylation/dephosphorylation pathways tightly control centrosome function.

Cyclins

Progression through the cell cycle is dependent on the interaction and activity of specific

cyclins and cyclin-dependent kinases (cdk). Until recently, it was unknown what role the cyclin/cdks played in the process of centrosome duplication because centrosomes will duplicate under experimental conditions in which cell cycle progression and DNA replication are blocked. This observation implied that these two processes can be uncoupled and suggested that they operated autonomously. However, three independent studies have reported that cyclinE/cdk2, whose activity peaks at the G_1/S transition precisely at initiation of centrosome duplication, is required for centrosome duplication [Hinchcliffe et al., 1999; Lacey et al., 1999; Matsumoto et al., 1999]. *Xenopus* embryos treated with cyclohexamide become blocked at DNA replication but the centrosomes continue through many rounds of duplication. When p21, a specific inhibitor of the activity of cdk2, was microinjected into the cyclohexamide-blocked embryos, centrosome duplication was also inhibited. When a 2× molar concentration of recombinant cyclinE was co-injected with p21, the inhibition of centrosome duplication was overcome. CyclinE/cdk2 activity is also required for the initial separation of the centriole pair, an early step in centriole duplication [Lacey et al., 1999]. As described above, centriole separation is also seen when Nek2 is overexpressed, this raises the possibility that these two kinases function within the same centrosome regulatory pathway.

Ran and Its Associated Proteins

Ran is a GTPase initially identified as a protein involved in the exchange of macromolecules across the nuclear envelope. GTP-bound Ran has recently been implicated in the control of microtubule nucleation. Depleting the pool of Ran's only known nucleotide exchange factor, RCC1, significantly impaired the ability of *Xenopus* sperm centrioles to nucleate microtubule asters in CSF arrested egg extracts [Ohba et al., 1999]. This inhibition was overcome by the addition of bacterially expressed human RCC1. A similar result is seen when a mutant Ran, T24N that only binds to GDP and inhibits the activity of RCC1, was added to the sperm centriole/egg extracts. A second Ran mutant, RanL43E, that functions to maintain Ran in its GTP bound state, not only induced aster formation around sperm centrioles but also resulted in the formation of asters that were centriole independent. These ectopic asters contained both NuMA and γ-tubulin. The ability of Ran activated asters to nucleate microtubules was dependent on the presence of a functional γ-tubulin ring complex [Wilde and Zheng, 1999]. How Ran, a nuclear transport protein, is involved in the regulation and organization of centrosomal microtubule arrays is not entirely clear. However, its predominate nuclear localization may restrict its activity to mitotic events that occur after nuclear envelope breakdown. Ran may target additional microtubule nucleating proteins to the centrosome, or it may directly regulate centrosomal microtubule nucleation or activate proteins already present at the centrosome. One such centrosomal protein that directly interacts with Ran is RanBPM, first identified as a Ran binding protein by two-hybrid analysis. Overexpression of RanBPM also results in ectopic microtubule nucleation [Nakamura et al., 1998]. RanBPM is localized to the centrosome throughout the entire cell cycle, unlike Ran, which is only present at the centrosome after nuclear envelope breakdown. It is therefore unlikely that Ran targets or shuttles RanBPM to the centrosome. Perhaps the function of Ran in centrosomal microtubule nucleation is mediated through the activity of RanBPM.

REGULATORY ACTIVITIES OF CENTROSOMES

Interphase Dynamics

The centrosome acts as the MTOC throughout all stages of the cell cycle. Microtubules have a defined structural polarity with their slow-growing, minus ends anchored at the centrosome. This organization imparts a structural polarity to the entire cytoskeleton with respect to the position of the centrosome. There is growing evidence that suggests the structural focus provided by the centrosome regulates many cellular processes. In normal resting cells, the centrosome resides in a perinuclear position and microtubules extend outward from this point toward the cell periphery. This organization allows for the correct placement of organelles that depend on microtubules for their proper function. The Golgi complex is one such organelle typically found closely associated with the centrosome and that relies on the presence of specific microtubule networks for proper vesicular trafficking. The position of the centrosome and its associated organelles may change in response to external stimuli. In the experimental model of wound healing in a monolayer

of tissue culture cells, the centrosome becomes repositioned to the side of the cell adjacent to the wound. When this centrosome movement is inhibited, cell movements involved in the wound healing process take a longer period of time [Ettenson and Gotlieb, 1992]. This indicates that proper centrosome positioning is required for optimal cell migration.

In migrating fibroblasts the position of the centrosome is dynamic. Following changes in the direction of cellular migration there is a subsequent repositioning of the centrosome and the cytoskeleton (Fig. 1). The centrosome continually repositions itself to a location between the nucleus and the leading edge of the cell [Schliwa et al., 1999]. Centrosome repositioning may play a role in the structural maintenance of the newly reorganized cytoskeleton. Thus by repositioning the centrosome, and subsequently its associated microtubule array, the cell can change the position and organization of cytoskeletal-associated organelles.

T lymphocytes (T cells) also display dynamic centrosome repositioning in response to contact with antigen-presenting cells (APCs). T cells continuously patrol the body for cells presenting foreign antigens in association with their MHC class II molecules. When a T cell comes in contact with an APC in an antigen-specific manner, it undergoes cytoskeletal rearrangements that result in the centrosome and its associated Golgi complex becoming repositioned adjacent to the area of contact between the two cells [Serrador et al., 1999]. This results in the polarized secretion of effector proteins directly to the area of contact. Similarly, centrosome repositioning and subsequent polarized secretion also takes place during T-helper cell activation of B cells and in the polarized section of perforin and granzymes by natural killer (NK) cells during contact with an appropriate target cell. Thus, a variety of immune functions operate though centrosome-directed polarization of the cytoplasm.

Mitotic and Postmitotic Centrosome Dynamics

Recently the role of centrosomes during mitosis has been reexamined. Originally, the centrosome itself was thought to act solely and directly to focus and regulate the microtubule bundle at each end of the bipolar spindle. But if the centrosome is essential for the proper formation and function of the bipolar spindle, how does one explain organisms, cells or in vitro systems that successfully form functional spindles in the absence of a centrosome [Heald et al., 1996; Smirnova and Bajer, 1992; Steffen et al., 1986]? Recent evidence indicates that the centrosome itself is not required for the generation of the focused microtubule ends of spindle poles during mitosis. This focusing activity can be carried out by a complex of scaffolding and motor proteins independent of a centrosome [Compton, 1998]. What, then, is the function of the centrosome during mitosis? Its activity as the regulator of microtubule nucleation ensures the presence of an adequate number of microtubules for the formation of a spindle. Also, the position of the centrosome at each spindle pole aids in the correct localization of many centrosomal proteins essential for regulating spindle dynamics. The centrosome may also play additional roles during mitosis. During interphase, the centrosome acts as a structural focus for microtubules and regulates the correct placement of cytoskeletal associated organelles. During mitosis, the centrosome may perform a similar function by acting as a structural anchor to correctly position the spindle poles with respect to the rest of the cell, the future position of the cleavage furrow and the extracellular environment. An example of this

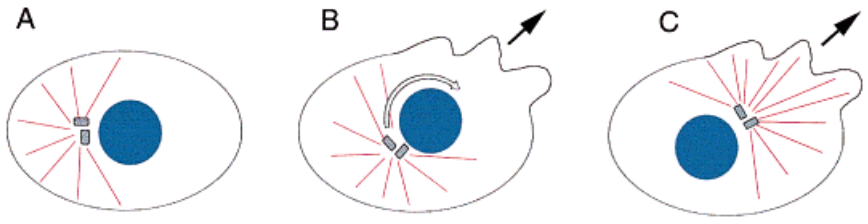

Fig. 1. Diagrammatic illustration of centrosome repositioning during cell migration. A resting fibroblast (**A**) begins to migrate in the direction of the black solid arrow (**B**). Subsequent to the initiation of cell migration, the centrosome moves to a position between the nucleus and the leading edge of the cell, as indicated by the gray arrow. The centrosome remains in this position as long as the cell maintains this direction of migration (C).

is seen in the early syncytial divisions of *Sciara coprophila* embryos. Normal fertilized embryos gain a centrosome by the male sperm, yet unfertilized (acentrosomal) embryos can still undergo the early stages of development [de Saint Phalle and Sullivan, 1998]. The unfertilized embryos nucleate microtubules from the condensed mitotic chromosomes and form a bipolar spindle. However, these spindles lack the astral and pole-to-pole microtubules normally nucleated and organized by the centrosome at the spindle poles. The resulting acentrosomal spindles become disorientated with respect to one another and their surroundings and subsequently fail to divide normally. This may be due to the absence of astral or pole to pole microtubules that are only established by the centrosome. These specific centrosomal microtubules appear to structurally regulate correct spindle position with respect to its environment.

During telophase, the centrosome acts as the structural focus of the cell during the postmitotic transition to the G_1 phase of the following cell cycle. During this period, the centrosome does not remain at the distal pole of each daughter cell but undergoes a dramatic relocation to a position adjacent to the intracellular bridge connecting the two daughter cells [Mack and Rattner, 1993]. This postmitotic relocation of the centrosome is dependent on the activity of the kinesin-like protein HsEg5 that functions to organize a distinct microtubule array essential for centrosomal movement [Whitehead and Rattner, 1998]. It is important to note that the interphase cytoskeleton does not form until the completion of this movement. This process may ensure that the cytoskeleton of each daughter cell generates a microtubule complex of opposite polarity [Mack and Rattner, 1993]. An additional function of this movement is the correct reorganization and positioning of the Golgi complex (Fig. 2). During mitosis, the Golgi complex breaks down into a randomly dispersed population of vesiculotubular elements. During anaphase, these elements become grouped at opposite sides of the cell, one group at the intracellular bridge and the other with the centrosome at the site of the spindle pole. When the centrosome repositions itself adjacent to the intracellular bridge it carries with it the Golgi elements that were originally at the spindle pole. This ensures that, at the end of mitosis, all the Golgi elements are adjacent to one another, where they can coalesce into a functional Golgi complex. This reorganization process is dependent on the correct movement and positioning of the centrosome. When postmitotic centrosome movement is abolished by the inhibition of HsEg5 activity, the Golgi complex fails to reorganize itself correctly [Whitehead and Rattner, 1998].

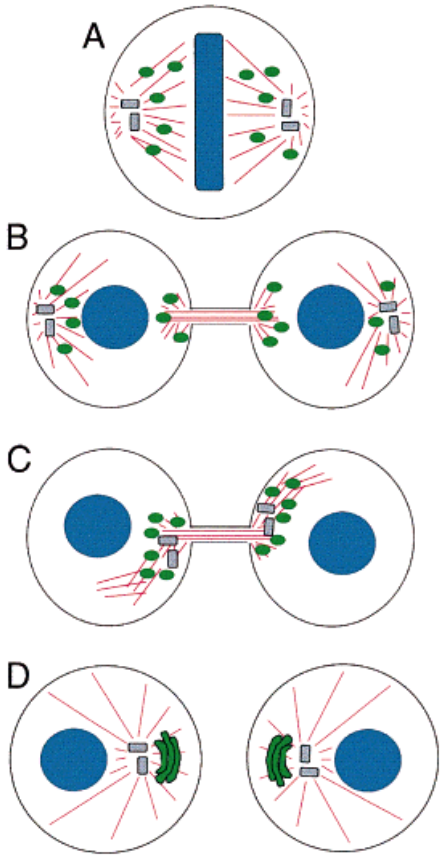

Fig. 2. Diagrammatic illustration of postmitotic centrosome and Golgi dynamics. **A:** The distribution of Golgi vesicles (green), chromosomes (blue), centrosomes (gray), and microtubules (red) in a metaphase tissue culture cell. Each centrosome is located at a spindle pole and Golgi vesicles are randomly dispersed. **B:** By early telophase, the intracellular bridge has formed and the Golgi vesicles are located at either end of the cell near the intracellular bridge or centrosome. **C:** During late telophase and early G_1, the centrosome and its associated Golgi vesicles, reposition themselves adjacent to the intracellular bridge. At this time, all the Golgi components reside in the same region of the cell. **D:** By late G_1, the intracellular bridge is no longer present between the two daughter cells and the Golgi complex has reformed.

Embryogenesis and Tissue Architecture

Embryonic development also depends on correct centrosome function and positioning. During embryogenesis, many specific factors are correctly distributed to particular daughter cells

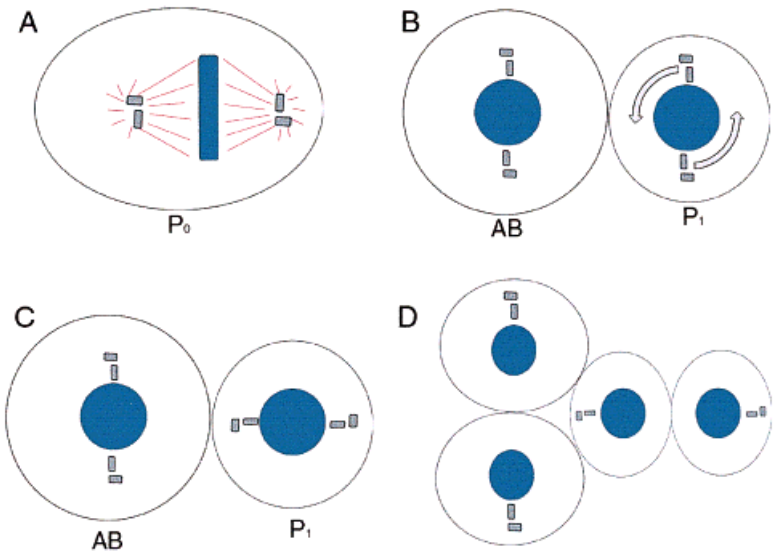

Fig. 3. Diagrammatic illustration of centrosome dynamics during early development of *Caenorhabditis elegans*. **A:** Centrosomes in the mitotic zygote (P_0) are disproportionally positioned with one being closer to the cell cortex that the other. This results in the formation of two daughter cells of unequal size, with the AB cell larger than the P_1. **B:** During the subsequent cell cycle, each centrosome has duplicated and migrated to opposite sides of the nucleus. After this initial movement the centrosomes of the P_1 cell undergo an additional 90° rotation as indicated by the gray arrows. **C:** This ensures that the mitotic spindles and cleavage furrows of the AB and P_1 cells will form in a perpendicular manner giving rise to the daughter cells (**D**)

by asymmetric cytokinesis. One striking example of how the centrosome plays an essential role in this process is during the initial cell divisions of *Caenorhabditis elegans* zygotes [Keating and White, 1998]. The first mitotic division of the zygote (P_0) is asymmetric and results in the formation of two cells of unequal size with one cell (AB) being larger than the other (P_1). This asymmetric division is due to the disproportionate position of the two centrosomes at the onset of the first mitotic division, i.e. one centrosome is positioned at the edge of the embryo while the other resides in a more central position (Fig. 3). During the subsequent division of the AB and P_1 cells, their cleavage furrows always form perpendicular to each other. Precise centrosome movements within the AB and P1 cells orchestrate this event. After the first mitotic division of the zygote, the centrosomes in the two daughter cells (AB and P_1) duplicate and migrate to opposite sides of their nuclei. Once this takes place, the centrosomes in the P_1 cell undergo an additional 90-dedree rotation, while the AB cell centrosomes do not. These centrosome movements ensure that the two subsequent cleavage furrows form in a perpendicular manner that is essential for the correct differentiation of the zygote.

Maintenance of correct tissue architecture requires that each individual cell be accurately positioned relative to its neighbor. This type of three-dimensional orientation requires directional polarity. In tissue cultured cells, this polarity appears to be regulated by the position of the centrosome. This tenant also holds true for cells within a tissue. One example of this is the organization of epithelial cells that form the lumen of the breast ducts. The centrosome in each luminal epithelial cell is positioned between the apical membrane and nucleus. This ensures that the secretory machinery associated with the cytoskeleton is located adjacent to the lumen. However, during the progression of breast cancer, there is a loss of both correct centrosome positioning and tissue architecture (Fig. 4). It is possible that the loss of tissue organization is a consequence of aberrant centrosome structure and position. Many recent reports have indicated that there may be a correlation between the acquisition of centrosomal abnormalities and the development of malignant phenotypes such as genomic instability and aneuploidy [Bischoff et al., 1998; Brinkley and Goepfert, 1998; Lingle et al., 1998; Lingle and Salisbury, 1999; Salisbury et al., 1999]. Ectopic expression of aurora2/STK15 (BTAK) kinase in 3T3 cells leads to abnormal centrosome number and transformation in vitro [Zhou et al., 1998]. Together, these observations suggest that the early acquisition of centrosome defects plays a direct role leading to the establishment of malignant phenotypes.

CONCLUDING REMARKS

Identification of components involved in centrosome function is beginning to open the "black box" of centrosome regulation that has both puzzled and inspired cell biologists since the

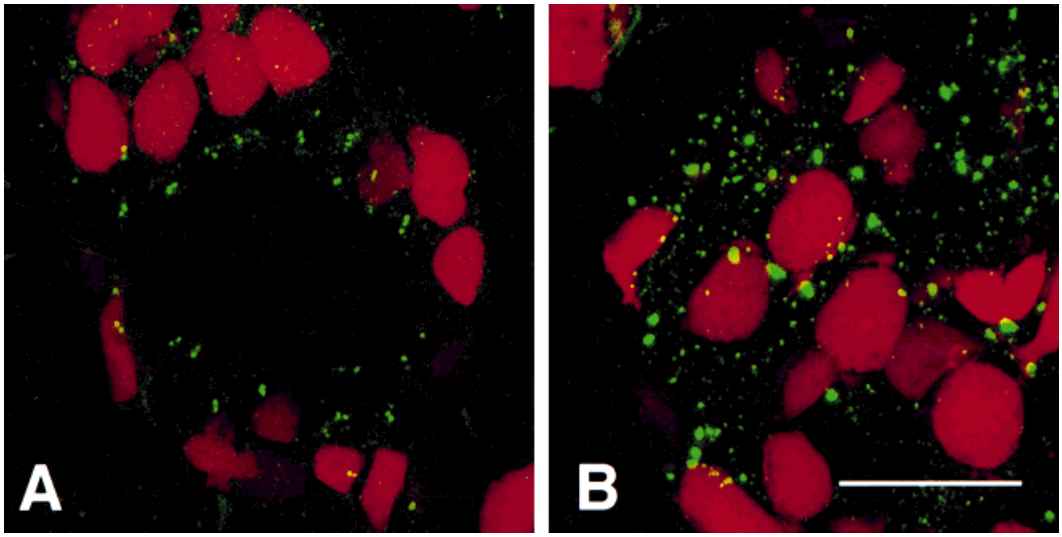

Fig. 4. Centriole distribution in normal and tumor breast tissue. **A:** A merged confocal projection of a normal human breast duct. The centrioles (green) are positioned between the nuclei (red) and the lumen of the duct. **B:** A human breast tumor displays a loss of normal tissue architecture and cell polarity. In tumor cells, the centrioles are abnormal in size, number, and position [Lingle et al., 1998]. Bar = 20 μM.

discovery of this organelle more than 100 years ago. However, many questions regarding centrosome structure and function remain. What signals initiate centriole duplication? Is centriole duplication a template driven process? How is centriole duplication regulated so that it only takes place once every cell cycle? What are the components regulating correct centriole structure and formation? What is involved in the maturation process that differentiates the older from the younger centriole? What are the signals that initiate and control proper centrosome repositioning? What targets a protein to the centrosome? What centrosomal proteins contribute to the acquisition of centrosomal defects seen in cancer?

REFERENCES

Andreassen PR, Lacroix FB, Villa-Moruzzi E, Margolis RL. 1998. Differential subcellular localization of protein phosphatase-1 alpha, gamma1, and delta isoforms during both interphase and mitosis in mammalian cells. J Cell Biol 141:1207–1215.

Bischoff JR, Anderson L, Zhu Y, Mossie K, Ng L, Souza B, Schryver B, Flanagan P, Clairvoyant F, Ginther C, Chan CS, Novotny M, Slamon DJ, Plowman GD. 1998. A homologue of *Drosophila aurora* kinase is oncogenic and amplified in human colorectal cancers. EMBO J 17:3052–3065.

Brinkley BR, Goepfert TM. 1998. Supernumerary centrosomes and cancer: Boveri's hypothesis resurrected. Cell Motil Cytoskeleton 41:281–288.

Compton DA. 1998. Focusing on spindle poles. J Cell Sci 111:1477–1481.

de Saint Phalle B, Sullivan W. 1998. Spindle assembly and mitosis without centrosomes in parthenogenetic *Sciara* embryos. J Cell Biol 141:1383–1391.

Earnshaw WC, Bernat RL. 1991. Chromosomal passengers: toward an integrated view of mitosis. Chromosoma 100:139–146.

Ettenson DS, Gotlieb AI. 1992. Centrosomes, microtubules, and microfilaments in the reendothelialization and remodeling of double-sided in vitro wounds. Lab Invest 66:722–733.

Fry AM, Mayor T, Meraldi P, Stierhof YD, Tanaka K, Nigg EA. 1998a. C-Nap1, a novel centrosomal coiled-coil protein and candidate substrate of the cell cycle-regulated protein kinase Nek2. J Cell Biol 141:1563–1574.

Fry AM, Meraldi P, Nigg EA. 1998b. A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators. EMBO J 17:470–481.

Heald R, Tournebize R, Blank T, Sandaltzopoulos R, Becker P, Hyman A, Karsenti E. 1996. Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* egg extracts. Nature 382:420–425.

Helps NR, Brewis ND, Lineruth K, Davis T, Kaiser K, Cohen PT. 1998. Protein phosphatase 4 is an essential enzyme required for organisation of microtubules at centrosomes in *Drosophila* embryos. J Cell Sci 111:1331–1340.

Hinchcliffe EH, Li C, Thompson EA, Maller JL, Sluder G. 1999. Requirement of Cdk2-cyclin E activity for repeated centrosome reproduction in **Xenopus** egg extracts. Science 283:851–854.

Keating HH, White JG. 1998. Centrosome dynamics in early embryos of *Caenorhabditis elegans*. J Cell Sci 111: 3027–3033.

Lacey KR, Jackson PK, Stearns T. 1999. Cyclin-dependent kinase control of centrosome duplication. Proc Natl Acad Sci USA 96:2817–2822.

Lingle WL, Salisbury JL. 1999. Altered centrosome structure in human breast tumors. Am J Pathol (in press).

Lingle WL, Lutz WH, Ingle JN, Maihle NJ, Salisbury JL. 1998. Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. Proc Natl Acad Sci USA 95:2950–2955.

Mack G, Rattner JB. 1993. Centrosome repositioning immediately following karyokinesis and prior to cytokinesis. Cell Motil Cytoskeleton 26:239–247.

Mack GJ, Rees J, Sandblom O, Balczon R, Fritzler MJ, Rattner JB. 1998. Autoantibodies to a group of centrosomal proteins in human autoimmune sera reactive with the centrosome. Arthritis Rheum 41:551–558.

Matsumoto Y, Hayashi K, Nishida E. 1999. Cyclin-dependent kinase 2 (Cdk2) is required for centrosome duplication in mammalian cells. Curr Biol 9:429–432.

Nakamura M, Masuda H, Horii J, Kuma K, Yokoyama N, Ohba T, Nishitani H, Miyata T, Tanaka M, Nishimoto T. 1998. When overexpressed, a novel centrosomal protein, RanBPM, causes ectopic microtubule nucleation similar to gamma-tubulin. J Cell Biol 143:1041–1052.

Ohba T, Nakamura M, Nishitani H, Nishimoto T. 1999. Self-organization of microtubule asters induced in *Xenopus* egg extracts by GTP-bound Ran. Science 284:1356–1358.

Paintrand M, Moudjou M, Delacroix H, Bornens M. 1992. Centrosome organization and centriole architecture: their sensitivity to divalent cations. J Struct Biol 108:107–128.

Rattner JB, Wang T, Mack G, Fritzler MJ, Martin L, Valencia D. 1992. MSA-36: a chromosomal and mitotic spindle-associated protein. Chromosoma 101:625–33.

Salisbury JL, Whitehead CM, Lingle WL, Barrett S. 1999. Centrosomes and cancer. Biol Cell 91:451–460.

Schliwa M, Euteneuer U, Graf R, Ueda M. 1999. Centrosomes, microtubules and cell migration. Biochem Soc Symp 65:223–231.

Schmidt PH, Dransfield DT, Claudio JO, Hawley RG, Trotter KW, Milgram SL, Goldenring JR. 1999. AKAP350, a multiply spliced protein kinase A-anchoring protein associated with centrosomes. J Biol Chem 274:3055–3066.

Serrador JM, Nieto M, Sanchez-Madrid F. 1999. Cytoskeletal rearrangement during migration and activation of T lymphocytes. Trends Cell Biol 9:228–233.

Smirnova EA, Bajer AS. 1992. Spindle poles in higher plant mitosis. Cell Motil Cytoskeleton 23:1–7.

Snaith HA, Armstrong CG, Guo Y, Kaiser K, Cohen PT. 1996. Deficiency of protein phosphatase 2A uncouples the nuclear and centrosome cycles and prevents attachment of microtubules to the kinetochore in *Drosophila* microtubule star (mts) embryos. J Cell Sci 109:3001–12.

Steffen W, Fuge H, Dietz R, Bastmeyer M, Muller G. 1986. Aster-free spindle poles in insect spermatocytes: evidence for chromosome-induced spindle formation? J Cell Biol 102:1679–1687.

Takahashi M, Shibata H, Shimakawa M, Miyamoto M, Mukai H, Ono Y. 1999. Characterization of a novel giant scaffolding protein, CG-NAP, that anchors multiple signaling enzymes to centrosome and the golgi apparatus. J Biol Chem 274:17267–17274.

Whitehead CM, Rattner JB. 1998. Expanding the role of HsEg5 within the mitotic and post-mitotic phases of the cell cycle. J Cell Sci 111:2551–2561.

Wilde A, Zheng Y. 1999. Stimulation of microtubule aster formation and spindle assembly by the small GTPase Ran. Science 284:1359–1362.

Witczak O, Skalhegg BS, Keryer G, Bornens M, Tasken K, Jahnsen T, Orstavik S. 1999. Cloning and characterization of a cDNA encoding an A-kinase anchoring protein located in the centrosome, AKAP450. EMBO J 18:1858–1868.

Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR, Sen S. 1998. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nature Genet 20:189–193.