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Dynamic Continuity of Nuclear and Mitotic Matrix Proteins in the Cell Cycle

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Abstract The eukaryotic cell nucleus is a membrane-enclosed compartment containing the genome and associated molecules supported by a highly insoluble filamentous network known as the nucleoskeleton or nuclear matrix. The nuclear matrix is believed to play roles in maintaining nuclear architecture and organizing nuclear metabolism. Recently, advances in microscopic techniques and the availability of new molecular probes have made it possible to localize functional domains within the nuclear matrix and demonstrate dynamic interactions between both soluble and insoluble components involved in the control of multiple nuclear transactions. Like the cytoplasm and its skeleton, the nucleoplasm is highly structured and very crowded with an equally complex skeletal framework. In fact, there is growing evidence that the two skeletal systems are functionally contiguous, providing a dynamic cellular matrix connecting the cell surface with the genome. If we impose cell cycle dynamics upon this skeletal organization, it is obvious that the genome and associated nuclear matrix must undergo a major structural transition during mitosis, being disassembled and/or reorganized in late G2 and reassembled again in daughter nuclei. However, recent evidence from our laboratory and elsewhere suggests that much of the nuclear matrix is used to form the mitotic apparatus (MA). Indeed, both facultative and constitutive matrix-associated proteins such as NuMA, CENP-B, CENP-F, and the retinoblastoma protein (Rb) associate within and around the MA. During mitosis, the nuclear matrix proteins may either become inert "passengers" or assume critical functions in partitioning the genome into newly formed G1 nuclei. Therefore, we support the view that the nuclear matrix exists as a dynamic architectural continuum, embracing the genome and maintaining cellular regulation throughout the cell cycle.

Key words: nuclear matrix, mitosis, mitotic apparatus, matrix-associated proteins, genome

> Yet, to the physiologist, the nucleus is a rather barren waste land; except the morphological changes the chromosomes undergo during cell division, it has been rather unapproachable experimentally.
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> C.P. Swanson [1957]

The eukaryotic cell nucleus has been a tough nut to crack in terms of its fine structure and functional 3-D organization. Not long ago, it was thought to be little more than a membrane-bound bag enclosing an amorphous nucleoplasm and sausage-like chromosomes. Fortunately, our insight into nuclear structure and genomic organization is improving. A large body of molecular, biochemical, and morphological data has accumulated in recent years attesting to the notion that most nuclear metabolism is highly compartmentalized both spatially and in terms of its solubility. The concept of an insoluble architecture is supported by the work from many laboratories describing within the nucleus an elaborate fibrogranular network, much like the cytoskeleton and referred to as the nucleoskeleton or nuclear matrix. This complex structure, comprised of protein, RNA, and some specific DNA, intersects with the nuclear lamina and forms an anastomosing network through the nucleus. Moreover, the nuclear matrix is juxtaposed, and perhaps functionally contiguous, with the cytoskeleton across the nuclear lamina and nuclear pores. These two skeletal systems appear to form a cell-wide matrix linking the cell surface to the genome. Historically, we are familiar with the cytoskeleton and its vast array of proteins but have only begun to grasp the nature and complexity of the nuclear matrix proteins. Though lacking information about the molecular composition of the matrix, we can, however, begin to visualize its architecture and

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speculate on its complex function in coordinating nuclear biology.

For example, there is growing recognition of matrix involvement in the organization of nuclear metabolic activity. It is upon the matrix that newly synthesized RNA and DNA are tightly associated at specific, albeit still poorly understood, regions. The need to elucidate how a myriad of biochemical events take place upon the matrix is underscored by recent morphological findings. Resinless section electron microscopy has allowed direct observation of matrix filaments uncovered by high- or physiological-salt extraction methods [He et al., 1990; Jackson and Cook, 1988] and draws rapt attention to the intricate architecture intimately associated with nuclear function. Perhaps the most compelling aspect of these structures is their ability to organize and reorganize during premitotic phases and during ballet-like maneuverings of chromosomes during mitosis. These morphological changes are not passive events, of course. There is ample evidence that some matrix proteins play significant roles in organizing chromosome associations on the mitotic spindle, thereby ensuring an equal partitioning of the genome to daughter cells. Immunofluorescence and resinless section electron microscopy of cells undergoing cell division support this concept. Furthermore, these approaches have provided an exquisite view of heretofore unseen architectural aspects of the mitotic apparatus that is comprised, in part, of proteins derived from the nuclear matrix [Capco and Penman, 1983; Wagner et al., 1986; Nickerson and Penman, 1992; He et al., 1995].

Taken together, advances in electron microscopy and high resolution light microscopy (supporting a new era of "molecular morphology") have revealed the nucleus to be far more than "a barren waste land" that is "unapproachable experimentally." Accordingly, during the past 40 years our impression of nuclear and cytoplasmic structure has changed quite perceptively [Penman, 1995]. However, an interminable, yet key, biological problem remains: how to integrate nuclear structure with function in a way that considers the architectural continuum throughout the cell cycle. The familiar pie-graph diagrams, present in most modern textbooks, that relegate phases of the cell cycle to variously sized wedges demeans the true metabolic continuum and underlying dynamic nuclear organization essential for the preparation and regulation of cell division. We have contemplated, from experimental data, the probable significance of the matrix in nuclear function; at present, however, we have only begun to speculate as to its fate and function during mitosis [He et al., 1995; Penman, 1995; Nickerson and Penman, 1992].

FACULTATIVE AND CONSTITUTIVE MATRIX PROTEINS

Direct observation of living cells during the course of a cell cycle easily conveys the need to incorporate "structural dynamics" into any model of nuclear architecture. Unfortunately, the structures made visible by various microscopic techniques have usually been of a static, and often misleading, nature. Through the use of cell cycle synchrony or differentiation protocols, identification and characterization of nuclear matrix and mitotic apparatus proteins has fostered the evolution of a paradigm that highlights their constitutive or facultative association with these structures [He et al., 1995]. In addition to studies emphasizing changes in 2-D gel profiles during the cell cycle and differentiation, we now have examples of specific proteins that are continuously matrix-bound (constitutive); in contrast, others have been identified as being transiently associated with the matrix at specific points of the cell cycle (facultative) [for review see He et al., 1995, and references therein]. Collectively, these data essentially affirm ideas that matrix composition is dynamic [Berezney, 1979]. Furthermore, several examples are now reported that demonstrate the construction of the mitotic apparatus (e.g., the spindle, kinetochore, midbody) utilizes both constitutive and facultative nuclear matrix proteins [Nickerson and Penman, 1992; Capco and Penman, 1983; Wagner et al., 1986; He et al., 1995].

NuMA AND THE SPINDLE MATRIX

Although the mitosis literature is replete with reference to a spindle matrix, little definitive information exists as to its molecular authenticity. Recent studies from our laboratory and elsewhere [reviewed in He et al., 1995] suggest that a dynamic continuity exists between proteins of the nuclear matrix and those of the mitotic apparatus. In fact, the concept that structural proteins could be involved in multiple functional roles from one phase in the cell cycle to the other is growing. The nuclear mitotic apparatus protein (NuMA) is a strong case in point. This

200–240 kD protein is constitutively associated with the matrix throughout interphase and then associates with the mitotic spindle during M phase. The extensive α-helical nature of NuMA probably results in the formation of a coiled-coil rod, similar to several structural proteins, and suggests the possibility of oligomerization. Conflicting experimental data currently leave no consensus on this subject [Harborth et al., 1995; Compton et al., 1993]. The identification of multiple, alternatively spliced NuMA isoforms [Tang et al., 1993], some of which can localize to RNA processing speckles and also immunoprecipitate snRNPs and reconstituted spliceosomes in vitro, suggest a separate interphase function as a structural interface for RNA processing [Zeng et al., 1994a]. Several other nuclear matrix antigens that localize to speckles have been specifically shown to interact with exon-containing mRNA [Blencowe et al., 1994]. Further supporting a structural role for NuMA, the anti-NuMA monoclonal antibody 2D3 can label a small subset of matrix core filaments [Zeng et al., 1994b]. The identification of NuMA as a MAR (matrix attachment region)-binding protein in vitro [Luderus et al., 1994] suggests an additional functional role for NuMA in the matrix. Although only a few NuMA antibodies label RNA processing sites specifically, all NuMA antibodies label the spindle poles at mitosis.

The role of NuMA during M phase itself is also likely to be manifold. The strategic localization with the spindle suggests a role in microtubule assembly, perhaps supporting nucleation and/or maintenance of chromosome-associated spindle fibers [He et al., 1995]. In support of this, microinjection of anti-NuMA antibodies [Yang and Snyder, 1992; Kallajoki et al., 1993] disrupts normal mitotic spindle formation and progression through mitosis [Gaglio et al., 1995]. In addition, overexpression experiments suggest that formation of daughter cell nuclei may require the presence of NuMA [Compton and Cleveland, 1993]. Conceptually, it is important to consider NuMA as an architectural matrix protein whose reorganization with cytoskeletal elements during M phase is an extension of its inherent molecular characteristics.

CENP-B

Another intriguing example of a constitutive nuclear matrix protein that associates with the mitotic apparatus involves the centromere-binding protein B (CENP-B) [Earnshaw et al., 1987]. CENP-B is localized as paired dots at the primary constriction in most species and is juxtaposed to the inner kinetochore plate. Moreover, CENP-B interacts with specific α-satellite centromeric DNA sequences [Masumoto et al., 1989; Muro et al., 1992; Casiano et al., 1993]. Salient to the discussion here, we have studied the dynamic cell cycle movements of prekinetochores using CREST antiserum (which primarily recognizes CENP-B [Brenner et al., 1981]). Despite the active nature of prekinetochore movements during interphase, we have found CENP-B/prekinetochores to be constitutively associated with the nuclear matrix core filaments [He et al., 1995]. At a cursory glance, these findings suggest a paradox: the nucleoskeleton, teeming with stable core filaments and fibrogranular assemblies, contains motile and dynamic organelles such as prekinetochores. The malleable characteristics of the prekinetochore are further appreciated using resinless section electron microscopy and CREST antisera to study the matrix preparations. Prekinetochore maturation includes a unique series of structural folding and unfolding during interphase. This includes a transformation from a tightly rounded ball with a central core in early G1, a stretched and extended form in late G1 loosened and unfolded during S phase, and finally, in G2, a pair of rounded structures that perfectly reflect the dimensions of the familiar paired dots seen by immunofluorescence [He et al., 1995].

The connection between interphase and mitotic structure is again borne out as CENP-B is associated with the chromosome scaffold during M phase. This suggests that when cells progress from interphase to mitosis and then back into G1, constitutive complexes within the underlying support system do not completely disassemble but remain part of a continuous architectural structure throughout the entire cell cycle. Further, the continuous association of a constitutive prekinetochore protein such as CENP-B with a structural matrix throughout interphase and mitosis suggests that dynamic movement and association of prekinetochores observed in nuclei of some cells (i.e., Indian and Chinese muntjac) may be facilitated, if not directed, by the insoluble nucleoskeletal architecture [He et al., 1995].

CENP-F

The list of transiently associated nuclear matrix proteins that are known to be part of the

mitotic apparatus is expanding [see He et al., 1995, and references therein]. CENP-F [Rattner et al., 1993; Casiano et al., 1993; Liao et al., 1995] and mitosin [Zhu et al., 1995a,b] are the most recently discovered components of the centromere/kinetochore complex. Sequence comparisons show that these large proteins are virtually identical [Zhu et al., 1995b; Liao et al., 1995]. For simplicity, they will be referred to hereafter as CENP-F. CENP-F transiently associates with the kinetochore [Rattner et al., 1993; Casiano et al., 1993; Liao et al., 1995; Zhu et al., 1995a,b], similar to CENP-E [Yen et al., 1991] and dynein [Zinkowski et al., 1991], two cytoplasmic proteins that move to the kinetochore following nuclear lamina dissolution. Unlike CENP-E and dynein, the ~350 kD CENP-F is a cell cycle–dependent nuclear phosphoprotein [Zhu et al., 1995b] that is not synthesized until the G1/S transition [Zhu et al., 1995b; Liao et al., 1995]. Most of the CENP-F protein pool partitions with and is widely distributed within the core filament matrix [Liao et al., 1995; Mancini, He, Brinkley, and Lee, unpublished observations]. Immunofluorescent labeling of CENP-F in core filament preparations excludes nucleoli and is not restricted to prekinetochores [Liao et al., 1995; Mancini, Brinkley, He, and Lee, unpublished observations]; at present, the ultrastructural localization of CENP-F within the core filament matrix is unknown. Although CENP-F is a facultative matrix-bound protein during half of interphase, it does not associate with prekinetochores until late G2 [Liao et al., 1995; Zhu et al., 1995b], coincident with an increase in its phosphorylation state [Zhu et al., 1995b]. The C-terminus of CENP-F has been shown to contain sequences required for nuclear localization, kinetochore targeting, and self-association [Zhu et al., 1995a]. At the beginning of cell division, a small subset of the CENP-F protein pool is detergent-resistant and found associated with the mitotic apparatus, first with the corona region of the kinetochore [Rattner et al., 1993; Zhu et al., 1995a]. Some CENP-F antibodies (e.g., polyclonal and monoclonal anti-mitosin antibodies) also label the mitotic spindle [Zhu et al., 1995b; He and Brinkley, unpublished]. As anaphase progresses, CENP-F eventually disassociates from the kinetochores, takes up residence at the spindle microtubule midzone, and associates with the midbody before a rapid degradation as M phase is completed [Zhu et al., 1995a; Liao et al., 1995]. As with CENP-B, the CENP-F antigen was first identified through the use of an autoimmune sera [Rattner et al., 1993; Casiano et al., 1993] that also led to its molecular cloning [Liao et al., 1995]; also, screening of bacterial expression libraries with the retinoblastoma protein, a G1-specific matrix-associated protein (see below), was used to identify "mitosin" clones [Shan et al., 1992; Zhu et al., 1995b].

In contrast to the constant matrix and kinetochore association of CENP-B, examination of CENP-F, and its facultative association with the matrix and kinetochore/mitotic apparatus, allows a picture to emerge that dramatically illustrates the dynamics of structural change (e.g., construction) that occurs during the cell cycle of this critical organelle. Kinetochore maturation and function, in fact, cannot easily be separated from cellular architecture at any point during the cell cycle. The constitutive components (CENP-B), or those which are added later (CENP-F), both spring from the same structural well.

THE RETINOBLASTOMA PROTEIN

The retinoblastoma protein (Rb) is a 110 kD nuclear phosphoprotein and prototypical tumor suppressor and cell cycle regulator [Riley et al., 1994]. Although a resident of the nucleus during all of interphase, Rb has been shown to specifically "tether to the nuclear structure" [Mittnacht and Weinberg, 1991] as a facultative nuclear matrix protein only during early G1 [Mancini et al., 1994]. It is during early G1 that Rb has been shown to act in regulating cell cycle progression [Goodrich et al., 1991]. The well-characterized cell cycle–dependent phosphorylation pattern of Rb correlates with its matrix association: only hypophosphorylated Rb during early G1 is capable of association with select fibrogranular assemblies, nucleolar remnants, and the nuclear lamina in core filament preparations [Mancini et al., 1994]. Hyperphosphorylated Rb does not bind nuclear structure, nor do mutant Rb proteins that are found in many tumors, indicating matrix interactions may be crucial for Rb function [Mittnacht and Weinberg, 1991; Durfee et al., 1994; Mancini et al., 1994]. This fits well with the idea that Rb can inhibit transcription factors like E2F that promote cell cycle progression [Riley et al., 1994; Hollingsworth et al., 1993], as several pieces of evidence suggest transcription takes place upon the matrix. In terms of its association with cell

architecture, the dynamic cell cycle–specific equilibrium of the Rb pool is obviously not required as a structural protein of the nucleoskeleton (e.g., Rb −/− cells are viable); however, the ability of Rb to influence the cell cycle appears to require this property for normal control of cell growth. At present, it is not known if two other Rb-like molecules, p107 [Ewen et al., 1991] and p130 [Hannon et al., 1993; Mayol et al., 1993; Li et al., 1993], share the ability to associate with the matrix.

The high number of putative Rb-associated proteins that have been cloned via several screening assays suggests promiscuity may be required for Rb function; moreover, cell cycle regulation during early G1 may not be its only functional role [Riley et al., 1994]. Interestingly, many of these Rb-binding proteins have been shown to be matrix- or mitotic apparatus–associated themselves, including lamins A and C [Mancini et al., 1994], p84 [Durfee et al., 1994], c-myc [Rustgi et al., 1991], hNUC [Chen et al., 1995] (hNUC is a human homologue of the yeast nucleoskeletal protein nuc2 [Hirano et al., 1988]), protein phosphatase 1 [Durfee et al., 1993], and mitosin/CENP-F (see above) [Zhu et al., 1995b]. Inactivation of Rb by several viral oncoproteins (SV40 large T antigen, adenovirus E1a protein, or human papilloma protein E7) is well documented, and all target the nuclear matrix as well [Deppert and Von Der Weth, 1990; Chatterjee and Flint, 1986; Greenfield et al., 1991]. One can envision these viral oncoproteins specifically targeting the matrix-bound Rb as a direct means to subvert cell cycle regulation. As Rb becomes dephosphorylated during mid to late mitosis [DeCaprio et al., 1992], the chromosome-specific localization of protein phosphatase type 1 [Fernandez et al., 1992], which can bind and presumably dephosphorylate Rb [Durfee et al., 1993; Alberts et al., 1993; Ludlow et al., 1993], may indicate that region-specific dephosphorylation of Rb occurs during M phase. Indeed, the observation that hypophosphorylated Rb partitions with isolated chromosome scaffolds is consistent with this idea [Mancini and Lee, unpublished]. These findings set a precedent for understanding how a nuclear protein can be regulated with respect to binding the matrix. Furthermore, these data support the possibility of multiple functions for Rb during the cell cycle controlled, perhaps, by its differential associations with components of cellular architecture.

CLOSING REMARKS

In most eukaryotic cells, the period of mitosis is only a brief interlude in the time required for a cell to complete the division cycle. Indeed, nuclear function and the complex organization of the interphase nucleus persists for 85–90% (or more) of the cell cycle. Perhaps it is inefficient for cells to completely dismantle and dissolve an elaborate nucleoskeleton only to reassemble it in daughter nuclei a few minutes later. Total dissolution seems not to occur in most cells; and some proteins, like NuMA, not only persist but carry out apparently novel functions unique and essential for the mitotic process. This phenomenon calls to mind an interesting parallel behavior in components of the cytoskeleton originally discovered by this laboratory. The proteins of the cytoplasmic microtubule complex (CMTC) of interphase disassemble as cells enter mitosis but are reassembled and organized into spindle microtubules that function uniquely to partition daughter chromosomes into daughter nuclei [Brinkley et al., 1975]. Thus, more than just providing a stable lattice in interphase, aspects of the nuclear matrix persist and function continuously, "embracing" the genome throughout the cell cycle.

These observations also appear to be in line with evolutionary considerations. Increasing amounts of comparative sequence data underscore the conservation of mitotic mechanisms at the molecular level. Examples of mitotic proteins conserved throughout eukaryotes include tubulins, kinesin-like motors, topoisomerase II, proteins of the DA-box family, and many others. This high conservation contrasts with the diverse cytology of mitotic events between evolutionarily distant phyla. Various versions of a "closed" mitosis, in which chromosome segregation occurs entirely within an intact nuclear envelope, are typical of lower eukaryotes and therefore likely constitute a more primitive type of mitotic architectural organization (Pickett-Heaps, 1974). In this type of mitosis, all components of the mitotic apparatus arise from, and are located within, the nucleus. We propose a hypothesis that the widespread involvement of interphase nuclear matrix proteins in the structure and function of the mitotic apparatus in higher eukaryotes reflects their common nuclear origin in more primitive life forms. In this scenario, evolution of mitotic mechanisms reflects architectural redistribution of conserved molecu-

lar components, rather than large-scale alterations of molecular and biochemical mechanisms. In a closed mitosis, therefore, nuclear matrix would be equivalent to mitotic matrix. In the more "evolved" open mitosis, a portion of the interphase nuclear matrix rearranges to become the "mitotic matrix," including the mitotic chromosome scaffold (topoisomerase II, SCII, CENP-B), mitotic spindle poles (NuMA), and other regions of the mitotic apparatus (reviewed by He et al., 1995). As more comparative molecular and immunocytochemical data for evolutionarily distant species become available, it will become possible to test and/or refine this hypothesis.

Elucidation of the mechanisms that support architecturally integrated nuclear and mitotic function(s) represent a logical next step now that we have some of the players identified. The anticipated stream of newly discovered genes (from "unrelated" library-screening projects) will undoubtedly add to the list of players whose protein products assert a structural role in either the nuclear matrix and/or the mitotic apparatus. Of course, many formidable challenges still remain: first, discerning how matrix components that comprise interphase and mitotic structures cooperate to partition the genome to daughter cells; second, understanding the nature and functional role of nonproteinaceous structural components (both DNA and RNA-containing); and, perhaps the most challenging, determining the nature of putative cell cycle checkpoint signaling through these structures. In each case, with increasing availability of molecular probes and ever-improving imaging technologies, we are now in the position to address these fundamental questions.

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REFERENCES

Alberts AS, Thorburn AM, Shenolikar S, Mumby MC, Feramisco JR (1993): Regulation of cell cycle progression and nuclear affinity of the retinoblastoma protein by protein phosphatases. Proc Natl Acad Sci USA 90:388–392.

Berezney RB (1979): Dynamic properties of the nuclear matrix. In: Busch H (ed): "The Cell Nucleus." San Diego, CA: Academic Press, Inc., pp 413–456.

Blencowe BJ, Nickerson JA, Issner R, Penman S, Sharp PA (1994): Association of nuclear matrix antigens with exon-containing splicing complexes. J Cell Biol 127:593–607.

Brenner S, Pepper D, Berns MW, Tan E, Brinkley BR (1981): Kinetochore structure, duplication, and distribution in mammalian cells: Analysis by human autoantibodies from scleroderma patients. J Cell Biol 91:95–102.

Brinkley BR, Fuller EM, Highfield DP (1975): Cytoplasmic microtubules in normal and transformed cells in culture: Analysis by tubulin antibody immunofluorescence. Proc Natl Acad Sci USA 72:4981–4985.

Capco DG, Penman S (1983): Mitotic architecture of the cell: The filament networks of the nucleus and cytoplasm. J Cell Biol 96:896–906.

Casiano CA, Landberg G, Ochs RL, Tan EM (1993): Autoantibodies to a novel cell cycle–regulated protein that accumulates in the nuclear matrix during S phase and is localized in the kinetochores and spindle midzone during mitosis. J Cell Sci 106:1045–1056.

Chatterjee PK, Flint SJ (1986): Partition of E1A proteins between soluble and structural fractions of adenovirus-infected and -transformed cells. J Virol 60:1018–1026.

Chen PL, Ueng YC, Durfee T, Chen KC, Yang-Feng T, Lee WH (1995): Identification of a human homologue of yeast nuc2 which interacts with the retinoblastoma protein in a specific manner. Cell Growth Differ 6:199–210.

Compton DA, Cleveland DW (1993): NuMA is required for the proper completion of mitosis. J Cell Biol 120:947–957.

Compton DA, Coulombe P, Cleveland DW (1993): Assembly of star-like oligomers of NuMA supports an anchoring role for NuMA in the interphase nucleus and at mitotic spindle poles. Mol Biol Cell 4S:80a.

DeCaprio JA, Furukawa Y, Ajchenbaum F, Griffin JD, Livingston DM (1992): The retinoblastoma-susceptibility gene product becomes phosphorylated in multiple stages during cell cycle entry and progression. Proc Natl Acad Sci USA 89:1795–1798.

Deppert W, Von Der Weth A (1990): Functional interactions of nuclear transport-defection simian virus 40 large T antigen with chromatin and nuclear matrix. J Virol 64: 838–846.

Durfee T, Becherer K, Chen PL, Yeh SH, Yang Y, Kilburn AE, Lee WH, Elledge SJ (1993): The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. Genes Dev 7:555–569.

Durfee T, Mancini MA, Jones D, Elledge SJ, Lee WH (1994): The amino-terminal region of the retinoblastoma gene product binds a novel nuclear matrix protein that co-localizes to centers for RNA processing. J Cell Biol 127: 609–622.

Earnshaw WC, Sullivan KF, Machlin PS, Cooke CA, Kaiser DA, Pollard TD, Rothfield NF, Cleveland DW (1987): Molecular cloning of cDNA for CENP-B, the major human centromere autoantigen. J Cell Biol 104:817–829.

Ewen ME, Xing YG, Lawrence JB, Livingston DM (1991): Molecular cloning, chromosomal mapping, and expression of the cDNA for p107, a retinoblastoma gene product–related protein. Cell 66:1155–1164.

Fernandez A, Brautigan DL, Lamb NJ (1992): Protein phosphatase type 1 in mammalian cell mitosis: Chromosomal localization and involvement in mitotic exit. J Cell Biol 116:1421–1430.

Gaglio T, Saredi A, Compton DA (1995): NuMA is required for the organization of microtubules into aster-like mitotic arrays. J Cell Biol 131:693–708.

Goodrich DW, Wang NP, Qian YW, Lee EY, Lee WH (1991): The retinoblastoma gene product regulates progression through the G1 phase of the cell cycle. Cell 67:293–302.

Greenfield I, Nickerson J, Penman S, Stanley M (1991): Human papillomavirus 16 E7 protein is associated with the nuclear matrix. Proc Natl Acad Sci USA 88:11217–11221.

Hannon GJ, Demetrick D, Beach D (1993): Isolation of the Rb-related p130 through its interaction with CDK2 and cyclins. Genes Dev 7:2378–2391.

Harborth J, Weber K, Osborn M (1995): Epitope mapping and direct visualization of the parallel, in-register arrangement of the double-stranded coiled-coil in the NuMA protein. EMBO J 14:2447–2460.

He DC, Nickerson JA, Penman S (1990): Core filaments of the nuclear matrix. J Cell Biol 110:569–580.

He DC, Zeng C, Brinkley BR (1995): Nuclear matrix proteins as components of the mitotic apparatus. Int Rev Cytol 162b:1–74.

Hirano T, Hiraoka Y, Yanagida M (1988): A temperature-sensitive mutation of the Schizosaccharomyces pombe gene nuc2+ that encodes a nuclear scaffold-like protein blocks spindle elongation in mitotic anaphase. J Cell Biol 106:1171–1183.

Hollingsworth RE Jr, Chen PL, Lee WH (1993): Integration of cell cycle control with transcriptional regulation by the retinoblastoma protein. Curr Opin Cell Biol 5:194–200.

Jackson DA, Cook PR (1988): Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. EMBO J 7:3667–3677.

Kallajoki M, Harborth J, Weber K, Osborn M (1993): Microinjection of a monoclonal antibody against SPN antigen, now identified by peptide sequences as the NuMA protein, induces micronuclei in PtK2 cells. J Cell Sci 104:139–150.

Li Y, Graham C, Lacy S, Duncan AM, Whyte P (1993): The adenovirus E1A-associated 130-kD protein is encoded by a member of the retinoblastoma gene family and physically interacts with cyclins A and E. Genes Dev 7:2366–2377.

Liao H, Winkfein RJ, Mack G, Rattner JB, Yen TJ (1995): CENP-F is a protein of the nuclear matrix that assembles onto kinetochores at late G2 and is rapidly degraded after mitosis. J Cell Biol 130:507–518.

Luderus ME, den Blaauwen JL, de Smit OJ, Compton DA, van Driel R (1994): Binding of matrix attachment regions to lamin polymers involves single-stranded regions and the minor groove. Mol Cell Biol 14:6297–6305.

Ludlow JW, Glendening CL, Livingston DM, DeCarprio JA (1993): Specific enzymatic dephosphorylation of the retinoblastoma protein. Mol Cell Biol 13:367–372.

Mancini MA, Shan B, Nickerson JA, Penman S, Lee WH (1994): The retinoblastoma gene product is a cell cycle–dependent, nuclear matrix–associated protein. Proc Natl Acad Sci USA 91:418–422.

Masumoto H, Masukata H, Muro Y, Nozaki N, Okazaki T (1989): A human centromere antigen (CENP-B) interacts with a short specific sequence in alphoid DNA, a human centromeric satellite. J Cell Biol 109:1963–1973.

Mayol X, Grana X, Baldi A, Sang N, Hu Q, Giordano A (1993): Cloning of a new member of the retinoblastoma gene family (pRb2) which binds to the E1A transforming domain. Oncogene 8:2561–2566.

Mittnacht S, Weinberg RA (1991): G1/S phosphorylation of the retinoblastoma protein is associated with an altered affinity for the nuclear compartment. Cell 65:381–393.

Muro Y, Masumoto H, Yoda K, Nozaki N, Ohashi M, Okazaki T (1992): Centromere protein B assembles human centromeric alpha-satellite DNA at the 17-bp sequence, CENP-B box. J Cell Biol 116:585–596.

Nickerson JA, Penman S (1992): Localization of nuclear matrix core filament proteins at interphase and mitosis. Cell Biol Int Rep 16:811–826.

Penman S (1995): Rethinking cell structure. Proc Natl Acad Sci USA 92:5251–5257.

Pickett-Heaps J (1974): The evolution of mitosis and the eukaryotic condition. BioSystems 6:37–48.

Rattner JB, Rao A, Fritzler MJ, Valencia DW, Yen TJ (1993): CENP-F is a ca. 400 kDa kinetochore protein that exhibits a cell-cycle dependent localization. Cell Motil Cytoskel 26:214–226.

Riley DJ, Lee EY, Lee WH (1994): The retinoblastoma protein: More than a tumor suppressor. Annu Rev Cell Biol 10:1–29.

Rustgi AK, Dyson N, Bernards R (1991): Amino-terminal domains of c-myc and N-myc proteins mediate binding to the retinoblastoma gene product. Nature 352:541–544.

Shan B, Zhu X, Chen PL, Durfee T, Yang Y, Sharp D, Lee WH (1992): Molecular cloning of cellular genes encoding retinoblastoma-associated proteins: Identification of a gene with properties of the transcription factor E2F. Mol Cell Biol 12:5620–5631.

Swanson CP (1957): "Cytology and Cytogenetics." Englewood Cliffs, NJ: Prentice-Hall, Inc.

Tang TK, Tang CJ, Chen YL, Wu CW (1993): Nuclear proteins of the bovine esophageal epithelium. II. The NuMA gene gives rise to multiple mRNAs and gene products reactive with monoclonal antibody W1. J Cell Sci 104:249–260.

Wagner B, Krochmalnic G, Penman S (1986): Resinless section electron microscopy of HeLa cell mitotic architecture. Proc Natl Acad Sci USA 83:8996–9000.

Yang CH, Snyder M (1992): The nuclear-mitotic apparatus protein is important in the establishment and maintenance of the bipolar mitotic spindle apparatus. Mol Biol Cell 3:1259–1267.

Yen TJ, Compton DA, Wise D, Zinkowski RP, Brinkley BR, Earnshaw WC, Cleveland DW (1991): CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. EMBO J 10:1245–1254.

Zeng C, He D, Berget SM, Brinkley BR (1994a): Nuclear-mitotic apparatus protein: A structural protein interface between the nucleoskeleton and RNA splicing. Proc Natl Acad Sci USA 91:1505–1509.

Zeng C, He D, Brinkley BR (1994b): Localization of NuMA protein isoforms in the nuclear matrix of mammalian cells. Cell Motil Cytoskel 29:167–176.

Zhu X, Chang KH, He D, Mancini MA, Brinkley BR, Lee WH (1995a): The C terminus of mitosin is essential for its nuclear localization, centromere/kinetochore targeting, and dimerization. J Biol Chem 270:19545–19550.

Zhu X, Mancini MA, Chang CF, Liu CY, Chen P, Shan B, Jones D, Yang-Feng TL, Lee WH (1995b): Characterization of a novel 350-kD nuclear phosphoprotein that is specifically involved in mitotic-phase progression. Mol Cell Biol 15:5017–5029.

Zinkowski RP, Meyne J, Brinkley BR (1991): The centromere-kinetochore complex: A repeat subunit model. J Cell Biol 113:1091–1110.