Journal of Cellular Biochemistry 63:268–279 (1996)

Remodeling of Nuclear Architecture During the Cell Cycle in *Drosophila* Embryos

Kristen M. Johansen, Jørgen Johansen, Kwang-Hyun Baek, and Ye Jin

Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011

Abstract Little is known about what determines the nuclear matrix or how its reorganization is regulated during mitosis. In this study we report on a monoclonal antibody, mAb2A, which identifies a novel nuclear structure in *Drosophila* embryos which forms a diffuse meshwork at interphase but which undergoes a striking reorganization into a spindle-like structure during pro- and metaphase. Double labelings with α-tubulin and mAb2A antibodies demonstrate that the microtubules of the mitotic apparatus co-localize with this mAb2A labeled structure during metaphase, suggesting it may serve a role in microtubule spindle assembly and/or function during nuclear division. That the mAb2A-labeled nuclear structure is essential for cell division and/or maintenance of nuclear integrity was directly demonstrated by microinjection of mAb2A into early syncytial embryos which resulted in a disintegration of nuclear morphology and perturbation of mitosis. © 1996 Wiley-Liss, Inc.

Key words: nuclear matrix, mitosis, *Drosophila* embryo, monoclonal antibody, spindle formation, nucleus

The interphase nuclear matrix consists of a complex network of core filaments and its associated proteins [Berezney and Coffey, 1977; Jackson and Cook, 1988; He et al., 1990]. During the various stages of the cell cycle this nuclear architecture undergoes significant reorganization, reflecting the major changes in the functional requirements of the nucleus during mitosis [Penman, 1995; Jackson, 1995]. These rearrangements include reorganization of the nucleoskeleton or nuclear matrix, disassembly/reassembly of the nuclear lamina, condensation/decondensation of the DNA, and assembly/disassembly of the mitotic apparatus. Although little is known about the behavior of the nuclear skeleton during division, the observation that core fibers are present at metaphase indicates that a fibrous nucleoskeletal network is also present during mitosis [Nickerson and Penman, 1992]. This suggests that at least a subset of the nucleoskeleton may not simply disassemble during mitosis, but may reorganize during the cell cycle and thus play an important structural role in transitions in nuclear morphology [Nickerson and Penman, 1992].

How the nuclear matrix is remodeled during division or what role it may play in nuclear reorganization events is not yet apparent in part because it has been difficult to isolate and define the composition of this scaffold. Recent efforts have focused on identifying and characterizing those molecules which operationally define the nuclear matrix, namely proteins which remain in the nucleus after nuclease digestions and detergent extractions [Berezney, 1991]. However, none of these proteins appear to constitute a major component of the core filaments [Jack and Eggert, 1992; Hozák et al., 1995], and thus it is not clear what structural role they may play. An alternate approach has employed the use of antibodies which stain the nuclear matrix obtained from autoimmune sera or which have been specifically generated against nuclear matrix fractions. EM-immunolocalization of some of these proteins has shown correlations with certain aspects of the nuclear matrix, for example localization to the dense assemblies [Nickerson and Penman, 1992; Wan et al., 1994; Zeng et al., 1994a] or to a subset of the core filaments [Nickerson and Penman, 1992; Zeng et al., 1994b]. One particularly striking example of a protein which redistributes during the cell cycle is NuMA (nuclear mitotic apparatus protein), a large coiled-coil nuclear matrix protein which relocates to the spindle poles at metaphase [Lydersen and Pettijohn, 1980; Yang et al., 1992; Compton et al., 1992]. Several studies

Received April 12, 1996; accepted April 29, 1996.

Address reprint requests to Kristen M. Johansen, Department of Zoology and Genetics, 3154 Molecular Biology Building, Iowa State University, Ames, IA 50011.

have shown that NuMA may be involved in spindle assembly [Kallajoki et al., 1991; Yang and Snyder, 1992; Compton and Luo, 1995; Gaglio et al., 1995] as well as in nuclear reassembly following division [Kallajoki et al., 1991, 1993; Compton and Cleveland, 1993]. However, in order to fully understand how the dynamic structure of the nucleus is organized and regulated, it is important that additional components of its structure be identified.

In the present study we have identified such a component using a monoclonal antibody, mAb2A, which labels *Drosophila* nuclei in a dynamic cell cycle-specific pattern. mAb2A identifies a nuclear meshwork at interphase which begins to reorganize into a spindle-like structure during prophase at a time when microtubules are excluded from the nucleus. At metaphase, however, the mAb2A-labeled spindle structure co-localizes with the mitotic microtubule spindle. The mAb2A-labeled spindle persists through anaphase, but begins to decondense during telophase exhibiting once again a meshwork-like staining pattern as in the interphase nucleus. This nuclear cytoarchitectural reorganization revealed by mAb2A suggests that it may be of functional importance during mitosis. In particular, the establishment of a spindle-like structure, the formation of which precedes or coincides with the assembly of the mitotic microtubule spindle, raises the possibility that this structure may be involved in the formation and/or function of the mitotic apparatus. A preliminary account of some of this work has been reviewed in Johansen [1996].

MATERIALS AND METHODS

Drosophila Embryo Collections

Wild-type *Drosophila melanogaster* Oregon R fly stocks were maintained according to standard protocols [Roberts, 1986]. Embryos were collected from either population cages or bottles using apple juice or molasses agar plates which had been spread with yeast.

Immunohistochemistry

For nuclear antibody labeling studies, embryos (0–3 h) were dechorionated in 50% Chlorox solution, washed with 0.7 M NaCl/0.2% Triton X-100, and fixed in a 1:1 heptane:fixative mixture for 20 min with vigorous shaking at room temperature. The fixative was either 4% paraformaldehyde in phosphate buffered saline (PBS) or Bouin's Fluid. Vitelline membranes were then removed either by the heptane-methanol method [Mitchison and Sedat, 1983] or by hand using a tungsten needle. Embryos were blocked in PBS with 1% normal goat serum (Cappel Organon Teknika, Durham, NC) and 0.4% Triton X-100. Primary antibody incubations using mAb2A (IgM) [Johansen, 1989], anti-α-tubulin monoclonal antibody (IgG1) (Sigma, St. Louis, MO), or anti-lamin monoclonal antibody (gift of Dr. P. Fisher, SUNY at Stony Brook) were performed either at room temperature or 4°C for 12–48 h. For single labelings, embryos were washed in PBS with 0.4% Triton X-100, incubated for 2.5 h with HRP-conjugated goat anti-mouse secondary antibody (1:200) (Bio-Rad, Richmond, CA), washed in PBS with 0.4% Triton-X100 followed by a rinse with PBS alone, and reacted for 5–10 min in PBS with 0.2 mg/ml diaminobenzidine (DAB) and 0.03% H_2O_2. Reactions were stopped by washing in PBS with 0.05% sodium azide and the samples mounted in 90% glycerol or were taken through an alcohol dehydration series, cleared in xylene, and mounted in DePeX (BDH). The labeled preparations were photographed on a Zeiss (Thornwood, NY) Axioskop using Ectachrome 64T film. The color positives were digitized using Adobe Photoshop and a Nikon Coolscan slide scanner. In Photoshop the images were image processed and in some cases converted to black and white before being imported into Freehand (Macromedia, San Francisco, CA) for composition and labeling.

For double and triple labelings by mAb2A together with α-tubulin or lamin antibodies and with Hoechst, embryos were collected, dechorionated, and pretreated for 20 s in heptane with 0.5 μM taxol before being fixed in 4% paraformaldehyde and devitellinized. Embryos were then sequentially incubated first with either α-tubulin antibody visualized with FITC-conjugated goat anti-mouse IgG1-specific antibody (Cappel) or with *Drosophila* lamin antibody visualized with FITC-conjugated goat anti-mouse IgG antibody (Cappel) and subsequently with mAb2A which was visualized using TRITC-conjugated goat anti-mouse IgM-specific antibody (Cappel). All antibody labelings were done in PBS buffer containing 0.4% Triton X-100 and 1% normal goat serum. For visualization of DNA, the antibody labeled embryos were incubated in 0.2 μg/ml Hoechst in PBS for 10 min. The final preparations were mounted in glycerol with 5% *n*-propyl gallate and viewed with a 100× Neo-

Fluor objective on a Zeiss Axioskop equipped with filter sets optimized and selective for each chromophore. Digital images were obtained using a high resolution cooled CCD camera (Paultek, Green Valley, CA), a PixelBuffer framegrabber (Perceptics, Knoxville, TN), and the NIH-Image software. The digital images were pseudocolored and imported into Photoshop where they were image enhanced and merged. Confocal microscopy was performed on a Nikon microscope equipped with a Noran Odessy real time confocal imaging system using a 60× objective. The images were acquired using the Image 1 software (Universal Imaging Corporation, West Chester, PA).

Antibody Perturbation Analysis

Antibody injection into 0–30 min syncytial embryos followed the procedures of Baek and Ambrosio [1995] except that embryos were aligned for lateral microinjection. Approximately 1 nl of either mAb2A or a control ascites fluid, both of which had been precleared by centrifugation, was injected into each embryo. The control ascites fluid was derived from the commercially available MOPC-104E mineral oil induced tumor cell line (Cappel) which produces mouse IgM antibody and which has the normal ascites fluid content of other mouse immunoglobulin and serum proteins. Embryos were allowed to develop for 3 h at 20°C after which they were fixed in Bouin's Fluid, hand devitellinized with a tungsten needle, and stained with Hoechst as described above to observe the nuclear DNA staining patterns. Egg collections were routinely divided into two samples to be used for either experimental or control injections in order to minimize variability between egg samples. Embryos were mounted in glycerol with 5% *n*-propyl gallate, viewed under epifluorescence as described above, and scored blind as either wild type, perturbed, or unfertilized. To test whether the difference in the distribution into these categories of experimental and control embryos was statistically significant, we performed a χ^2-test of the null hypothesis that the two distributions were different.

RESULTS

mAb2A Labeling of Embryonic Nuclei Shows a Dynamic Cell-Cycle Specific Distribution Pattern

The monoclonal antibody 2A was originally identified as an IgM-secreting clone from a hybridoma fusion with a synthetic peptide derived from extracellular *Notch* sequence as an immunogen; however, the mAb2A does not recognize the Notch protein [Johansen, 1989]. Instead mAb2A specifically labels a novel nuclear component which shows a dynamic cell cycle-specific distribution pattern. However, since the mAb2A is of the IgM subtype and does not recognize the

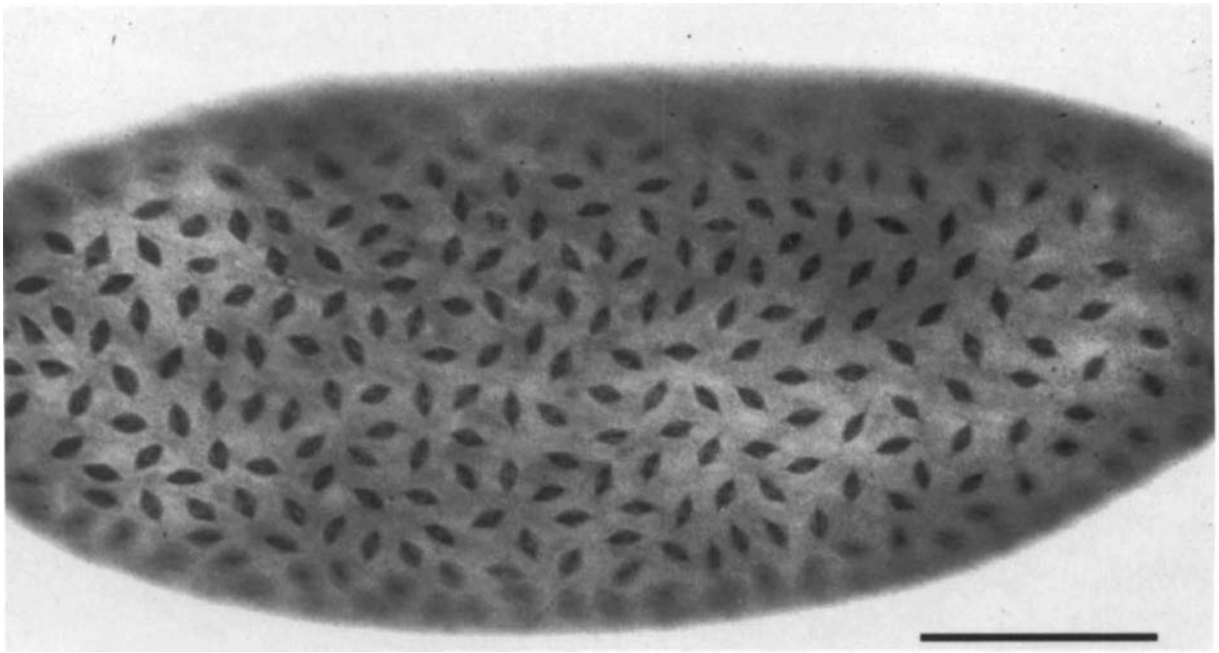

Fig. 1. Whole-mount preparation of a pre-cellular blastoderm *Drosophila* embryo demonstrating labeling by mAb2A of synchronous metaphase nuclei. Scale bar = 75 μm.

denatured antigen on immunoblots a direct determination of the biochemical nature of this component has yet to be completed [Walker et al., 1995; Johansen, 1996] and will be presented elsewhere. Consequently, the main emphasis of this report is to provide an immunohistochemical analysis of the nuclear cytoarchitectural reorganization revealed by mAb2A and on the possible functional importance of nuclear matrix remodeling for nuclear division.

Figure 1 shows a pre-cellular blastoderm stage *Drosophila* embryo which has been stained with the mAb2A, demonstrating staining in all of the synchronized metaphase somatic nuclei. At these early stages the nuclear components and mitotic spindles are large and easily visualized, facilitating the analysis of their interactions. Nuclear staining by mAb2A has been observed in all tissues analyzed thus far, including imaginal discs, salivary glands, larval muscle cells, and ovaries (data not shown). Figure 2 shows differential interference contrast (DIC) images of *Drosophila* embryonic nuclei that demonstrate the striking redistribution of mAb2A nuclear labeling during various stages of the cell cycle. At interphase (Fig. 2A) the antibody identifies a diffuse meshwork of labeled structures throughout the nucleus, which can be followed by focusing in different optical planes. In addition, it appears that mAb2A labeling may be associated with the nuclear envelope at this stage although this has not been ascertained directly (Fig. 2A–C, arrows). During prophase the labeled nuclear meshwork undergoes a pronounced reorganization and begins to condense within the nucleus (Fig. 2B,C) and aligns to form a distinct spindle-like structure (Figs. 2D, 3A). Intriguingly, this structure begins to form during prophase at a time when the nuclear envelope is still intact and when tubulin is excluded from entering the nucleus [Fuchs et al., 1983; Hiraoka et al., 1990]. The mAb2A-labeled spindle-like structure continues to be labeled during anaphase (Fig. 2E) but begins to decondense during telophase (Fig. 2F,G), exhibiting once again a meshwork-like

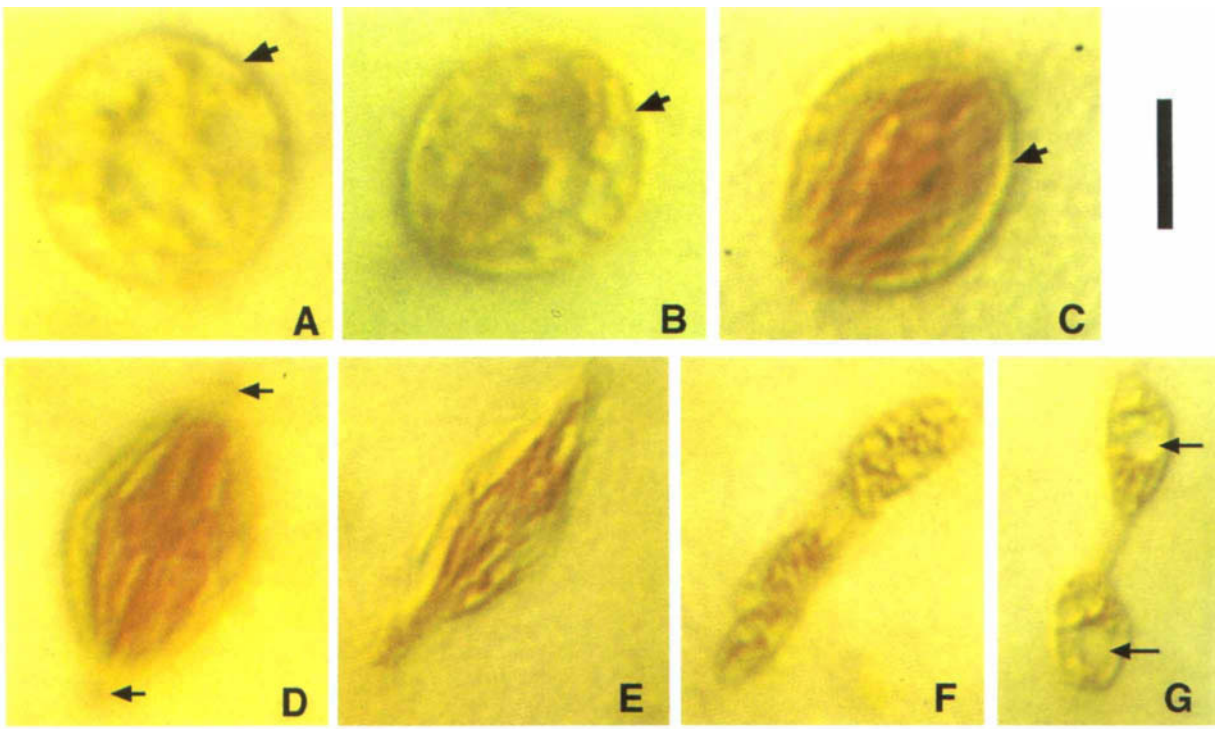

Fig. 2. *Drosophila* embryo nuclei labeled by mAb2A from various stages of the cell cycle: **(A)** interphase; **(B)** prophase; **(C)** prometaphase; **(D)** metaphase; **(E)** anaphase; **(F)** early telophase; **(G)** late telophase. At prophase (B) the diffuse meshwork labeled by mAb2A in interphase (A) begins to undergo a condensation which leads to the labeling of a spindle-like structure at metaphase (D). At metaphase the duplicated centrosomes also appear to be labeled by the antibody (arrows). The spindle-like structure continues to be labeled during anaphase (E) before decondensation and redistribution of the mAb2A labeling during telophase (F,G). During interphase (A), prophase (B), and prometaphase (C), mAb2A labeling also appears to be associated with the nuclear envelope (arrows). The mAb2A labeling appears to be excluded, however, from the reassembling nucleoli (G, arrows). Scale bar = 5 μm.

staining pattern which does not include the nucleolus (Fig. 2G, arrows).

The Nuclear Structure Identified by mAb2A Forms a True Spindle at Metaphase

In order to address whether the spindle-like structure stained by mAb2A during metaphase (Figs. 1, 2D) is a true spindle, we performed confocal microscopy of mAb2A-labeled nuclei which were visualized using a fluorescently tagged secondary antibody. Optical sectioning of metaphase nuclei showed that the mAb2A-labeled structure clearly could be resolved as being organized into separate and distinct spindle domains and thus that it is not a solid structure as it may appear under DIC and conventional fluorescent optics (Fig. 3A). In addition, confocal microscopy revealed the presence of a fibrous network of mAb2A staining located at the metaphase plate (Fig. 3A, arrow). The mAb2A labeled spindle-like structure remains intact during anaphase (Fig. 2E) when the sister chromatids segregate and the microtubules disassemble as shown in Figure 3B and C. Thus it appears that mAb2A identifies a novel spindle-like structure which may be involved in the formation and/or function of the mitotic apparatus.

Formation of the mAb2A-Labeled Spindle

In order to ascertain the relative genesis of the spindle-like structure identified by mAb2A to that of the microtubule spindle apparatus and to analyze whether the two structures are coincident at metaphase, we performed triple label-

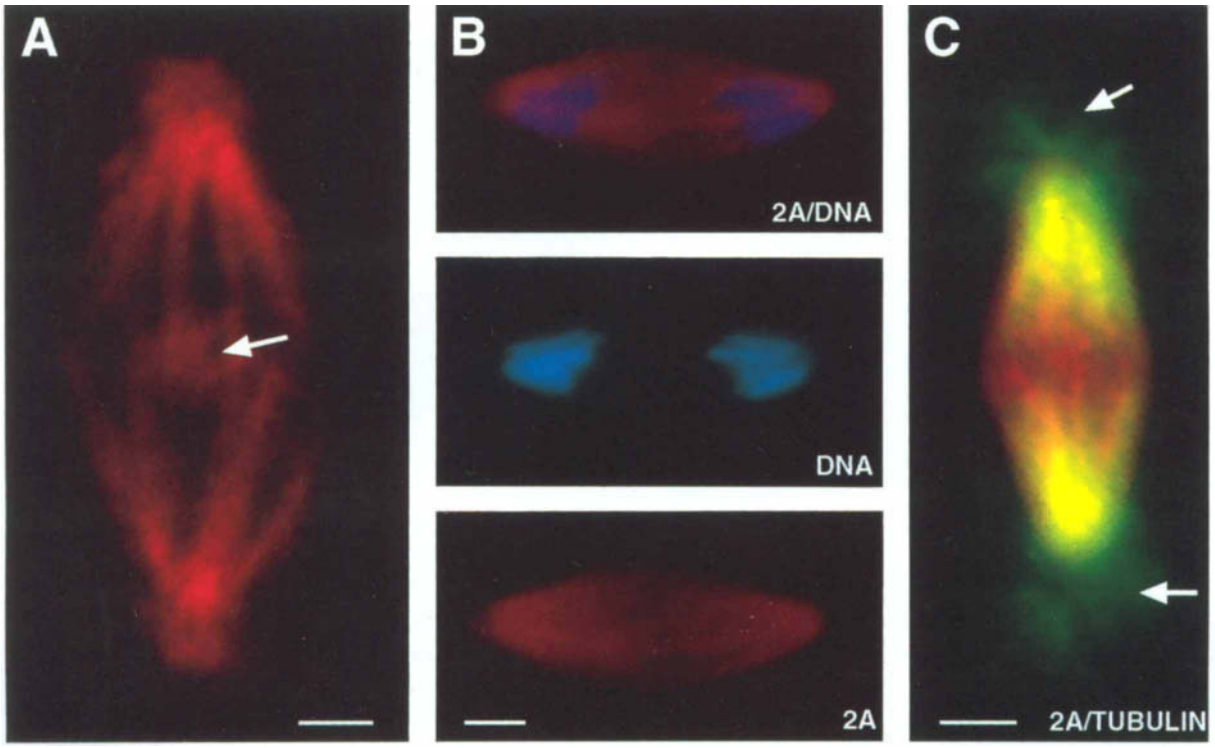

Fig. 3. mAb2A labels a spindle-like structure at metaphase and anaphase. **A:** Optical section obtained by confocal microscopy with a 60× objective of a metaphase nucleus labeled with mAb2A and visualized using TRITC-conjugated secondary antibody. The figure shows that the mAb2A labeling is of a true spindle and not of a solid structure as may appear under DIC optics. In addition, the confocal analysis revealed the presence of a mAb2A labeled fibrous network located at the metaphase plate (arrow). The embryo was fixed in Bouins's fluid. Scale bar = 1.5 μm. **B:** Double labeling of an anaphase nucleus with mAb2A and with Hoechst which labels DNA. The chromosomes are drawn to opposite poles while mAb2A continue to label a spindle-like structure. The top panel shows the composite staining pattern of both mAb2A and Hoechst while the panels below show the individual stainings pseudocolored in blue for Hoechst and in red for mAb2A. Scale bar = 2 μm. **C:** Double labeling of an anaphase nucleus labeled with a mAb to α-tubulin (pseudocolored green) and with mAb2A (pseudocolored red). At this stage the microtubules are in the process of shortening and disassembly while the mAb2A labeled scaffold continues to extend from centrosome to centrosome. mAb2A labeling co-localizes with anti α-tubulin antibody labeling at the polar regions of the spindle (yellow) whereas the asters are labeled by anti α-tubulin antibody only (green, arrows) and not by mAb2A. Scale bar = 2 μm. Embryos in B and C were stabilized with taxol and fixed in 4% paraformaldehyde.

ings with mAb2A using anti-α-tubulin antibody to visualize the microtubules and Hoechst staining to visualize the DNA (Fig. 4A). Figure 4A shows exposures through different filter sets optimized for each chromophore used in the same optical plane. Mitosis in *Drosophila* is considered to be "closed," although in fact the nuclear envelope does disassemble at metaphase [Stafstrom and Staehelin, 1984; Fuchs et al., 1983; Hiraoka et al., 1990]. This is demonstrated in Figure 4B where metaphase nuclei are triple labeled with mAb2A, Hoechst and an mAb to *Drosophila* lamin as a marker for the nuclear envelope [Maus et al., 1995]. In the composite picture (Fig. 4B) it can be clearly seen that the spindle poles extend beyond the nuclear envelope, which has partly broken down providing an entry point for the microtubule fibers to extend towards the metaphase plate and to form the spindle apparatus. Thus, at prophase and prometaphase the mAb2A labeled spindle structure is already being formed (Figs. 2B,C, 4A) while the tubulin microtubule spindles only start to assemble during prometaphase when nuclear envelope breakdown is initiated (Fig. 4A). These results clearly suggest that formation of the mAb2A-labeled spindle begins before microtubule aggregation. However, we could not resolve whether completion of the mAb2A-labeled spindle precedes or coincides with microtubule spindle assembly. At metaphase the labeling of mAb2A and anti α-tubulin antibody is congruent except for the microtubule asters which are not labeled by mAb2A (Figs. 3C, 4A). Centrosomes and in some cases individual spindle fibers could be resolved as labeled by both mAb2A and anti α-tubulin antibody (bright yellow color in the composite panel of Fig. 4A). However, by telophase the mAb2A labeling again redistributes into a diffuse meshwork (Fig. 2F,G) which overlaps with the Hoechst staining as the chromatin also decondenses (Fig. 4A).

Injection of mAb2A Into Syncytial Embryos Perturbs Normal Nuclear Division and Blocks Development

The immunolocalization studies described above using mAb2A demonstrate the existence of a nuclear component that reorganizes during mitosis and may be involved in nuclear architectural remodeling during the cell cycle. Its dynamic redistribution pattern suggests that this component may play an essential role in nuclear structure and/or nuclear division. In order to test this hypothesis, we performed perturbation experiments by injecting antibody into syncytial stage 2 *Drosophila* embryos. The fertilized egg undergoes 13 rapid and nearly synchronous nuclear divisions giving rise to about 6,000 nuclei before cell boundaries form after 3 h of development [Zalokar and Erk, 1976; Foe and Alberts, 1983], thus affording excellent antibody access to the dividing nuclei. Experimental embryos were injected with mAb2A ascites fluid while control embryos were injected with the same amount of commercially available IgM-containing (MOPC-104E) ascites which recognize an antigen not detected in *Drosophila*. Injected embryos were allowed to develop for 3 h at 20°C, after which they were fixed in Bouin's fluid and stained with Hoechst to compare the nuclear DNA staining patterns.

The phenotypes of representative stage 12 mAb2A-perturbed embryos are shown in Figure 5A–D. In general, many fewer nuclei are observed in the experimental than in the control-injected embryos (Fig. 5E,F), and in some extreme cases only a few nuclear remnants can be identified in the mAb2A injected embryos. Whereas by this stage all of the somatic nuclei in control embryos have migrated to the periphery (Fig. 5E,F), nuclei in mAb2A-perturbed embryos can be observed at many planes of focus throughout the embryo, indicating that the remaining nuclei either failed to migrate to the periphery or failed to remain at the periphery once there. Besides being fewer in number, the surviving nuclei in mAb2A-perturbed embryos are morphologically aberrant and appear to be disintegrating (Fig. 5D, arrowheads and inset). This contrasts with the organized array of nuclei seen in MOPC-104E ascites injected control embryos which are indistinguishable from wild type embryos of this stage (Fig. 5F). Thus mAb2A injection into early embryos clearly affects nuclear integrity and perturbs normal embryo development and mitosis. This suggests that function of the nuclear scaffold identified by mAb2A is essential for nuclear structure and for normal division to occur.

In order to address whether these results were statistically significant we compared the distribution of perturbed, wild type, and unfertilized embryos from two groups of embryos injected with mAb2A and MOPC-104E ascites, respectively (Table I). In both control and experimental embryos, 25% of the eggs were unfertilized and failed to undergo any nuclear divisions.

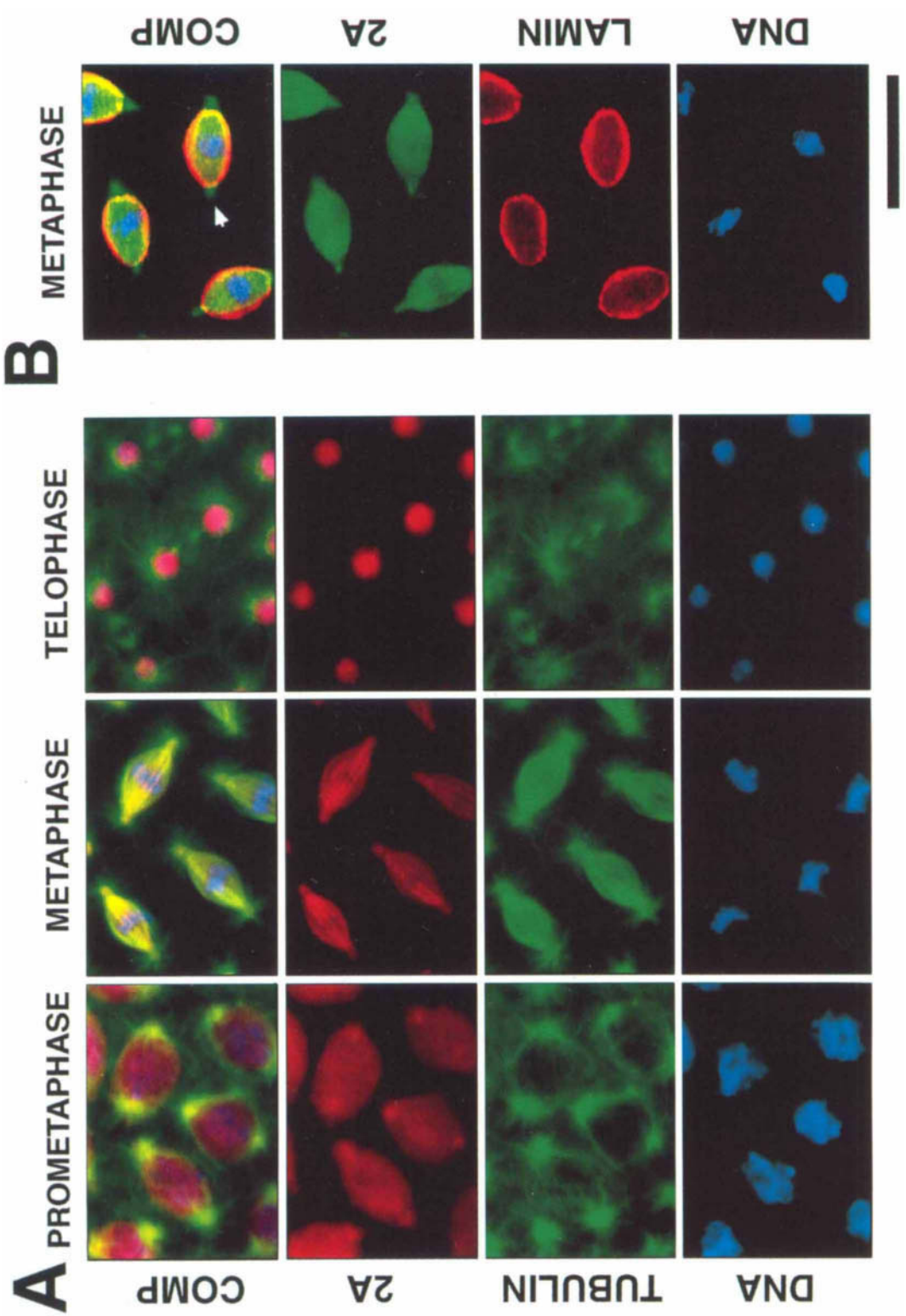
A
PROMETAPHASE
METAPHASE
TELOPHASE
B
METAPHASE
COMP
2A
TUBULIN
DNA
COMP
2A
LAMIN
DNA

Figure 4.

The remaining 75% of injected eggs were scored for nuclear phenotype, either as "wild-type" indicated by development to the pre-cellular or cellular blastoderm stage with synchronous, normally stained nuclei (Fig. 5E,F) or "perturbed" indicated by abnormal nuclear number, morphology, and localization (Fig. 5A–D). Embryos were scored blind to minimize bias. In the mAb2A-injected embryos, 55.3% appeared perturbed and 19.7% appeared wild-type, while in the control-injected embryos only 4.3% appeared perturbed and 70.7% appeared wild-type (Table I). Thus there was a marked difference in distributions of phenotypes between experimental- and control-injected embryos. Application of a χ^2-test demonstrated that the distribution of the mAb2A-injected embryos is significantly different from that of the control distribution ($P < 0.005$), supporting the conclusion that injection of mAb2A perturbs nuclear integrity and/or mitosis.

DISCUSSION

In this study we report on the labeling by the mAb2A of a novel nuclear component which dynamically redistributes during the cell cycle in *Drosophila* appearing as a nuclear meshwork at interphase but as a spindle-like structure at meta- and anaphase. At present there are two possible ways to interpret the nuclear labeling we have observed. It could reflect a redistribution of the antigens recognized by mAb2A from the nuclear matrix at interphase to the mitotic spindle at metaphase, or alternatively it could reflect the actual structural reorganization of a nuclear matrix component stained by mAb2A during the cell cycle. However, since our data from double and triple labelings demonstrate that the mAb2A-stained spindle-type structure begins forming in prophase and prometaphase at a time when tubulin is still excluded from the nucleus, we favor a model in which the mAb2A-stained nuclear meshwork itself reorganizes during the cell cycle [Johansen, 1996]. This meshwork forms a diffuse scaffolding at interphase overlapping with the DNA staining pattern, but reorients during prophase to establish a spindle-like lattice, which at this stage is distinct from the microtubules. However, at metaphase it is clear that the mAb2A-labeled spindle co-localizes with the mitotic microtubule spindle, an observation consistent with the hypothesis that it may be necessary for the assembly and/or maintenance of the mitotic apparatus. Optical sectioning of labeled metaphase nuclei by confocal microscopy confirmed that the mAb2A-labeled structure does form a true spindle. In addition, it demonstrated the presence of filamentous staining at the metaphase-plate reminiscent of nuclear matrix core filaments as have been resolved previously by resin-less electron microscopic imaging [Nickerson and Penman, 1992]. This nuclear cytoarchitectural reorganization revealed by mAb2A staining during the cell cycle suggests that it may play an important role both in defining functional domains within the interphase nucleus as well as in the establishment and function of the mitotic apparatus.

That the mAb2A-labeled nuclear structure is essential for cell division and/or maintenance of nuclear integrity was directly demonstrated by microinjection of mAb2A into early syncytial embryos which resulted in a disintegration of nuclear morphology and perturbation of mitosis. However, the observed antibody blocking of nuclear division and embryo development may arise from one or more of several possible scenarios. The interference by mAb2A with nuclear division could be a direct effect of blocking proteins involved in spindle assembly and/or function which are necessary for mitosis. Alternatively, perturbation of the mAb2A-labeled meshwork at interphase could lead to disintegration of nuclear structure, which as a secondary

Fig. 4. Comparison of mAb2A labeling of nuclei to the localization of DNA, α-tubulin, and *Drosophila* lamin. **A:** Triple labelings of nuclei with mAb2A (red), Hoechst (blue), and an mAb to α-tubulin (green) at prometaphase, metaphase, and telophase. All pictures at each stage are from the same optical plane. The composite staining is shown in the top row; the individual contributions from the mAb2A, anti α-tubulin antibody, and Hoechst staining patterns are shown below. At prometaphase the mAb2A labeled spindle-like structure can be seen to be in the process of forming prior to the establishment of the microtubule spindle fibers. At this stage, the microtubules still cap the nuclei, and are only just starting to assemble into spindles. At metaphase the staining patterns of mAb2A and of anti α-tubulin antibody are coincident except for the astral microtubules which stain only with anti α-tubulin antibody. In contrast, at telophase the mAb2A labeling is completely dissociated from the microtubule staining pattern. Instead the mAb2A labeling is confined to the reassembling nucleus whereas the microtubules are forming midbodies and astral fibers. **B:** Metaphase nuclei triple labeled with mAb2A (green), Hoechst (blue), and with an mAb to *Drosophila* lamin (red) which serves as a marker for the nuclear envelope [Maus et al., 1995]. The composite (top panel) demonstrates that the mAb2A labeled structure, which originally formed inside the intact nuclear envelope (see Fig. 2B,C), at this stage extends outside the nuclear envelope, which, although still present, has partially broken down at the ends (arrow). Scale bar = 15 μm.

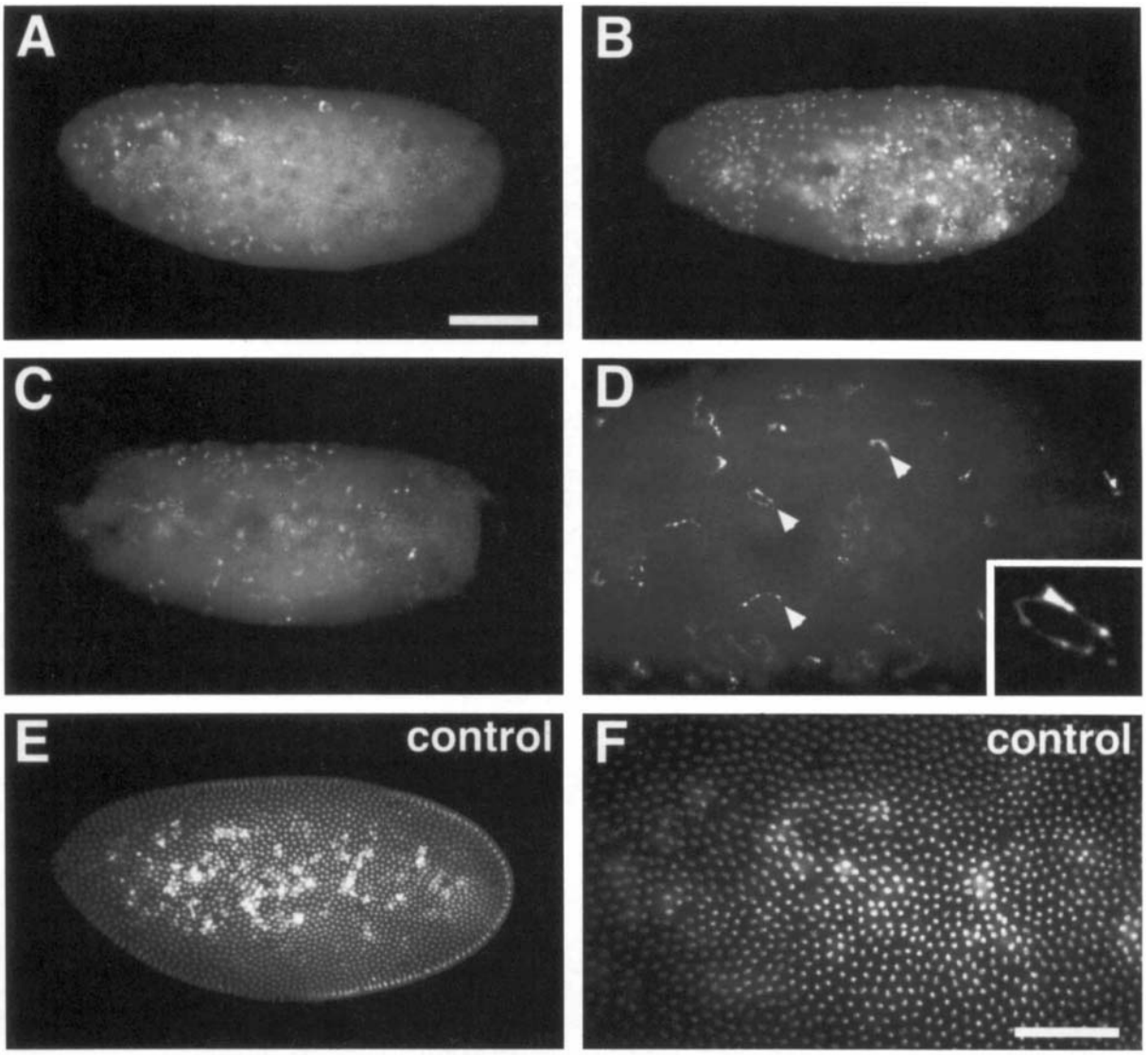

Fig. 5. Injection of mAb2A results in abnormal nuclear morphology and perturbs nuclear division. Syncytial *Drosophila* embryos (0–30-min-old) were injected with either mAb2A ascites fluid (A–D) or a control IgM-containing ascites (MOPC-104E) (E,F), allowed to develop for 3 h at 20°C, fixed, and the resulting phenotype assayed by labeling the nuclei with Hoechst. **A–C:** Three representative embryos displaying the consequences for nuclear phenotype of perturbation by mAb2A. Embryos show a marked reduction in total numbers of nuclei, and the remaining nuclei show abnormal morphology and, in many cases, failure to migrate to or remain at the periphery. Scale bar = 100 μm. **D:** Higher magnification of embryo in C, demonstrating the "disintegration" phenotype of the mAb2A perturbed nuclei which is marked by filamentous Hoechst staining (arrows and inset). **E:** Control embryo injected with MOPC-104E ascites shows wild-type Hoechst staining pattern. **F:** Higher magnification of embryo in E shows the normal highly ordered Hoechst staining pattern of nuclei at the periphery typical of wild-type embryos. Scale bar = 20 μm.

consequence would prevent nuclear divisions from occurring. Another possibility is that antibody binding to the interphase meshwork may provide sterical hindrances to changes in nuclear architecture during the cell cycle. To address whether one or a combination of these possibilities occurs it will be necessary to molecularly characterize the nuclear component labeled by mAb2A. Although at present we cannot determine the exact mechanism, it is nevertheless clear from the current perturbation analysis that interference with the mAb2A-labeled structure during the cell cycle has severe consequences for nuclear integrity and division.

The nuclear meshwork staining at interphase observed using mAb2A and its reorganization beginning at prophase into a true spindle structure at metaphase is especially interesting in the

TABLE I. Injection of mAb2A Into Early Embryos Perturbs Nuclear Integrity and Division*

	n	Perturbed	Wildtype	Unfertilized
Control embryos (%)[a]	92	4 4.3%	65 70.7%	23 25.0%
mAb2A injected embryos (%)[b]	76	42 55.3%	15 19.7%	19 25.0%

*Embryos were injected with either control or experimental antibodies 30 min after being laid and then incubated at room temperature for 2 h before fixation. The development of the embryos and nuclear integrity were assessed and scored based on Hoechst labeling of the embryos (see Fig. 5). Application of a χ^2-test shows that the distribution of the mAb2A injected embryos is different from that of the control distribution ($P < 0.005$). Since the frequency of unfertilized embryos is the same in both experiments, we interpret these results to show that mAb2A injection perturb normal nuclear integrity and division.

[a]Control embryos were injected with IgM-containing ascites fluid derived from the MOPC-104E cell-line.

[b]Experimental embryos were injected with mAb2A ascites fluid.

context of the "spindle matrix" hypothesis [Porter, 1976; McIntosh, 1981; Pickett-Heaps et al., 1982; Pickett-Heaps, 1986; Forer and Wilson, 1994]. This model proposes that there exists an elastic microtrabecular lattice within the nucleus which extends throughout the mitotic spindle and which serves to organize spindle function by coordinating the interactions between the lattice, the microtubules, and other essential spindle proteins, such as kinesin [McIntosh, 1981; Pickett-Heaps et al., 1982, 1984; Picket-Heaps, 1986]. Considerations of the forces operating on the mitotic spindle have also led to the suggestion of the existence of structural elements necessary for maintaining the metaphase spindle [Fuller and Wilson, 1992]. Evidence in support of such a structural matrix which could play a direct role in spindle function has come from experiments which have demonstrated (1) the continued poleward movement of chromosomes at anaphase even after severing the kinetechore microtubule fibers [Forer, 1966; Wilson and Forer, 1988]; (2) that the spindle remains organized even when the centrosome is removed, suggesting it contains a minus-end organizing function [Nicklas et al., 1989]; and (3) that both plus and minus-ends of the spindle microtubules remain free to exchange tubulin subunits, implying that the microtubules alone are not acting as a stable scaffold but are in constant flux [Mitchison, 1989; Sawin and Mitchison, 1991, 1994]. Although the spindle matrix has yet to be molecularly defined, the redistribution pattern of NuMA to the pericentriolar regions of the mitotic spindle [Yang and Snyder, 1992; Compton and Cleveland, 1993; Compton and Luo, 1995; Gaglio et al., 1995] has given rise to the suggestion that NuMA may constitute such a spindle matrix component [Yang and Snyder, 1992; Gaglio et al., 1995]. Our observations of mAb2A labeling is also consistent with the existence of a nuclear spindle matrix structure. The results presented here provide evidence that mAb2A may identify a previously undetected nuclear matrix component which dynamically reorganizes to form a stable spindle-like scaffold during mitosis which does not contract at anaphase, but which stays intact while the chromosomes are pulled to the poles. Consequently, the properties of this scaffold suggest it could comprise an essential stabilizing element for microtubule spindle formation and for the forces generated by the mitotic spindle apparatus during chromosome segregation. Future work aimed towards the molecular characterization of this scaffold structure and elucidation of its function and regulation promises to provide significant new insight into nuclear matrix reorganization and coordination of cell cycle specific processes.

ACKNOWLEDGMENTS

The authors thank Ms. Anna Yeung for expert technical assistance, Ms. Virginia Lephart for maintenance of fly stocks, and Dr. P. Fisher for his generous gift of the *Drosophila* lamin antibody. We also thank Dr. Raj Lartius for help and assistance with the confocal microscopy. This work was supported by NIH grant GM 50906 and NSF grant DCB-9009312 to K.M.J. Journal Paper No. J-17080 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 3429 and supported by Hatch Act and State of Iowa funds.

REFERENCES

Baek K-H, Ambrosio L (1995): An efficient method for microinjection of mRNA into *Drosophila* embryos. BioTechniques 17:1024–1026.

Berezney R (1991): The nuclear matrix: A heuristic model for investigating genomic organization and function in the cell nucleus. J Cell Biochem 47:109–123.

Berezney R, Coffey DS (1977): Nuclear matrix: Isolation and characterization of a framework structure from rat liver nuclei. J Cell Biol 73:616–637.

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

Compton DA, Luo C (1995): Mutation of the predicted $p34^{cdc2}$ phosphorylation sites in NuMA impair the assembly of the mitotic spindle and block mitosis. J Cell Sci 108:621–633.

Compton DA, Szilak I, Cleveland DW (1992): Primary structure of NuMA, an intranuclear protein that defines a novel pathway for segregation of proteins at mitosis. J Cell Biol 116:1395–1408.

Foe V, Alberts BM (1983): Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. J Cell Sci 61: 31–70.

Forer A (1966): Characterization of the mitotic traction system, and evidence that birefringent spindle fibers neither produce nor transmit force for chromosome movement. Chromosoma 19:44–98.

Forer A, Wilson P (1994): A model for chromosome movement during mitosis. Protoplasma 179:95–105.

Fuchs J-P, Giloh H, Kuo C-H, Saumweber H, Sedat JW (1983): Nuclear structure: Determination of the fate of the nuclear envelope in *Drosophila* during mitosis using monoclonal antibodies. J Cell Sci 64:331–349.

Fuller MT, Wilson PG (1992): Force and counterforce in the mitotic spindle. Cell 71:547–550.

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.

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

Hiraoka Y, Agard DA, Sedat JW (1990): Temporal and spatial coordination of chromosome movement, spindle formation, and nuclear envelope breakdown during prometaphase in *Drosophila melanogaster* embryos. J Cell Biol 111:2815–2828.

Hozák P, Sasseville AM-J, Raymond Y, Cook PR (1995): Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. J Cell Sci 108:635–644.

Jack RS, Eggert H (1992): The elusive nuclear matrix. Eur J Biochem 209:503–509.

Jackson DA (1995): Nuclear organization: Uniting replication foci, chromatin domains and chromosome structure. BioEssays 17:587–591.

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

Johansen KM (1989): Identification and localization of the gene product of the *Notch* locus of *Drosophila melanogaster*. Doctoral thesis, Yale University, New Haven, CT.

Johansen KM (1996): Dynamic remodeling of nuclear architecture during the cell cycle. J Cell Biochem 60:289–296.

Kallajoki M, Weber K, Osborn M (1991): A 210 kDa nuclear matrix protein is a functional part of the mitotic spindle: A microinjection study using SPN monoclonal antibodies. EMBO J 10:3351–3362.

Kallajoki M, Herborth 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.

Lydersen BK, and Pettijohn DE (1980): Human-specific nuclear protein that associates with the polar region of the mitotic apparatus: Distribution in a human/hamster hybrid cell. Cell 22:489–499.

Marshall WF, Dernburt AF, Harmon B, Agard DA, Sedat JW (1995): Specific interactions of chromatin with the nuclear envelope in Drosophila. Mol Biol Cell 6:121a.

Maus N, Stuurman N, Fisher PA (1995): Disassembly of the *Drosophila* nuclear lamina in a homologous cell-free system. J Cell Sci 108:2027–2035.

McIntosh JR (1981): Microtubule polarity and interaction in mitotic spindle function. In Schweiger HG (ed): "International Cell Biology 1980–1981." Berlin: Springer-Verlag, pp 359–368.

Mitchison TJ (1989): Poleward microtubule flux in the mitotic spindle: Evidence from photoactivation of fluorescence. J Cell Biol 109:637–652.

Mitchison T, Sedat J (1983): Localization of antibody determinants to whole *Drosophila* embryos. Dev Biol 99:261–264.

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

Nicklas RB, Lee GM, Rieder CL, Rupp G (1989): Mechanically cut mitotic spindles: Clean cuts and stable microtubules. J Cell Sci 94:415–423.

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

Pickett-Heaps JD (1986). Mitotic mechanisms: An alternative view. Trends Biochem Sci 11:504–507.

Pickett-Heaps JD, Tippit DH, Porter KR (1982): Rethinking mitosis. Cell 29:729–744.

Pickett-Heaps J, Spurck T, Tippet D (1984): Chromosome motion and the spindle matrix. J Cell Biol 99:137s–143s.

Porter KR (1976): Motility in cells. In Goldman CR, Pollard T, Rosenbaum J (eds): "Cell Motility." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp 1–28.

Price CM, Pettijohn DE (1986): Redistribution of the nuclear mitotic apparatus protein (NuMA) during mitosis and nuclear assembly. Exp Cell Res 166:295–311.

Roberts DB (1986): Basic *Drosophila* care and techniques. In Roberts DB (ed): "Drosophila, a Practical Approach." Oxford: IRL Press pp 1–38.

Stafstrom JP, Staehelin LA (1984): Dynamics of the nuclear envelope and of nuclear pore complexes during mitosis in the *Drosophila* embryo. Eur J Cell Biol 34:179–189.

Sawin KE, Mitchison TJ (1991): Poleward microtubule flux in mitotic spindles assembled in vitro. J Cell Biol 112:941–954.

Sawin KE, Mitchison TJ (1994): Microtubule flux in mitosis is independent of chromosomes, centrosomes, and antiparallel microtubules. Mol Biol Cell 5:217–226.

Walker DL, Jin Y, Irwin P, Dong H, Johansen J, Johansen KM (1995): mAb2A, which labels a nuclear scaffold, identifies two novel nuclear antigens. Mol Biol Cell 6:423a.

Wan KM, Nickerson JA, Krockmalnic G, Penman S (1994): The B1C8 protein is in the dense assemblies of the nuclear matrix and relocates to the spindle and pericentriolar filaments at mitosis. Proc Natl Acad Sci USA 91:594–598.

Wilson P, Forer A (1988): Ultraviolet microbeam irradiation of chromosomal spindle fibres shears microtubules and permits study of the new free ends in vivo. J Cell Sci 91:455–468.

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.

Yang CH, Lambie EJ, Snyder M (1992): NuMA: An unusually long coiled-coil related protein in the mammalian cell nucleus. J Cell Biol 116:1303–1317.

Zalokar M, Erk I (1976): Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster*. J Microsc Biol Cell 25:97–106.

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 Cytoskeleton 29:167–176.