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PROSPECTS

Dynamic Remodeling of Nuclear Architecture During the Cell Cycle

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Abstract The nuclear matrix is an integral part of nuclear structure which undergoes a profound reorganization during the cell cycle reflecting major changes in functional requirements. This includes the processes of DNA replication and gene expression at interphase and partitioning of the nuclear contents during mitosis. Using a monoclonal antibody (mAb2A) which specifically stains a novel nuclear meshwork which reorganizes during the cell cycle in *Drosophila*, we have initiated a study to: 1) more closely analyze this structural reorganization; 2) clone and characterize the antigens recognized by this antibody; and 3) isolate other interacting proteins in order to gain insight into the regulation of this process. The mAb2A-labeled structure changes from what appears as a diffuse meshwork at interphase to a distinct spindle-like scaffold at prophase. Since at metaphase the microtubules of the mitotic apparatus co-localize with the mAb2A spindle structure, a model is considered whereby the nuclear mAb2A-labeled scaffolding reorganizes during the cell cycle to provide a guide for the establishment of the mitotic apparatus. The mAb2A has identified two separate antigens, each of which shows similar distribution patterns. One of these antigens has been partially cloned and contains an unusual tandem ser-thr kinase domain. The association of this kinase homologue with a nuclear scaffold which reorganizes during the cell cycle suggests that it may be involved in regulating changes in nuclear architecture during the cell cycle and/or in mediating the downstream consequences of such changes.

Key words: nuclear scaffold, spindle formation, monoclonal antibody, mitosis, kinase, *Drosophila*

Whereas for many years the nucleus was thought to consist simply of chromatin, the nucleolus, and nucleoplasm confined to a specialized region by a nuclear envelope, these being the only structures prominent using conventional EM microscopy, recent advances in probing the structure of the nucleus have made it clear that the organization of the nucleus is far more complex [Berezney and Coffey, 1977; Jackson and Cook, 1988; He et al., 1990]. Particularly, nuclear organization has been found to be intricately interrelated with gene expression, cell division, and cell differentiation [Getzenberg et al., 1991a; Stein et al., 1994; Sahlas et al., 1993; Ingber, 1993]. However, the structural and molecular basis for this organization, often referred to as the nuclear matrix or nucleoskeleton, has been difficult to define. As an early definition, it tended to be considered whatever biological material remained after numerous extractions and digestions [reviewed in Berezney, 1991], leading many to doubt that it truly existed as a real and functional entity. However, as microscopic imaging techniques have improved demonstrating a discrete scaffold structure [Berezney and Coffey, 1977; Jackson and Cook, 1988; He et al., 1990], as correlations between the component matrix proteins observed and the state of cell differentiation or transformation have been made [Getzenberg et al., 1991b; van Wijnen et al., 1993], and as more and more researchers examining gene expression from the level of regulation of transcription have found specific and functional interactions with matrix attachment regions (MAR's) or scaffold attachment regions (SAR's) [Laemmli et al., 1992], considerable evidence for the existence of such a nuclear structure has been provided.

Consequently, recent efforts have focused on identifying and characterizing the molecules which make up and associate with the nuclear matrix with the aim of understanding how the nucleus is organized and what regulates its structural changes at different phases of the cell cycle. Such studies will ultimately enable the

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synthesis of an integrative model for how gene expression is regulated, what controls cell division, and how a cell is able to respond to cues from its environment. Although relatively few matrix molecules have thus far been identified which would be candidates for playing such a functional role, there are a number of antigens which have been localized to discrete domains in the interphase nucleus suggesting an association with a nuclear matrix. For example, this kind of organization has been observed in the specific "speckle" patterns obtained with various nuclear antigen antibodies [O'Keefe et al., 1994; Wu et al., 1994; Bregman et al., 1994; Sahlas et al., 1993], in the organization of domains of transcription and splicing [Xing et al., 1993; Zhang et al., 1994], in the specific association of splicing domains with the nuclear matrix [Blencowe et al., 1994; Zeng et al., 1994], and in the detection of specific channels or tracks for some mRNA and proteins [Zachar et al., 1993; Meier and Blobel, 1992].

Dynamic Reorganization of the Nucleus During Mitosis

During cell division, the complex interphase nuclear structure has to be duplicated and reorganized [reviewed in Murray and Hunt, 1993] implying major dynamic structural changes in the organization of the entire nucleus. Some of these changes, such as the condensation of DNA into metaphase chromosomes and nuclear lamina disassembly occurring as a consequence of phosphorylation, have been carefully analyzed in a number of studies [Saitoh and Laemmli, 1994; Gerace and Blobel, 1980], but as yet due to lack of probes very little work has been oriented towards analyzing the reorganization events of the structural elements of the nucleus. However, there are a number of molecules which show mitosis-specific redistribution patterns including the CENPs and the INCENPs [reviewed in Earnshaw and Mackay, 1994]. One particularly striking example of a protein which redistributes during the cell cycle is NuMA (**Nu**clear **M**itotic **A**pparatus protein), a protein which was originally identified as a nuclear matrix protein which localizes to the spindle poles at metaphase [Lydersen and Pettijohn, 1980]. NuMA encodes a very large (~230kD) protein consisting of unique head and tail domains with a large internal coiled-coil domain [Compton et al., 1992; Yang and Snyder, 1992] which is predicted to form a two-stranded coiled-coil structure [Parry, 1994]. Immunolocalization as well as antibody perturbation studies have demonstrated that NuMA plays a functional role in mitosis and nuclear structure [Compton and Cleveland, 1993; Yang and Snyder, 1992; Kallajoki et al., 1993]. Although there may be differential localization of alternatively spliced isoforms of NuMA [Tang et al., 1994], immunoEM labeling has revealed that at least one isoform can be ultrastructurally localized to the nuclear matrix [Zeng et al., 1994]. Thus, NuMA is a strong candidate for a nuclear matrix core filament molecule which may play a structural role in the organizational changes of the nucleus as it advances through the cell cycle.

In addition, resin-less EM imaging techniques used to visualize interphase nuclear matrix core filaments have been applied to metaphase cells, revealing an intriguing filamentous network surrounding and interconnecting the chromosomes lined up at the metaphase plate [Nickerson and Penman, 1992]. This suggests that at least some of the nuclear matrix core filaments may structurally reorganize rather than dissociate at metaphase, and thus raises the prospects for a functional role of the nuclear matrix in mitosis as well. However, whether the nuclear matrix is simply a nuclear constituent to be apportioned during nuclear division or whether it actively guides the division process has not been established.

mAb2A Identifies a Nuclear Scaffold Which Dynamically Reorganizes During the Cell Cycle in *Drosophila*

Studying the reorganization events which occur during mitosis promises to yield significant new information in two essential areas of cell biology: 1) cell cycle control, since cell division is critically dependent on major changes in nuclear architecture; and 2) the role of nuclear organization in nuclear function, since new probes and approaches will enable a more rigorous functional analysis. Thus, in order to better understand how the dynamic structure of the nucleus is organized and regulated, it is important that additional components of this structure be identified as well as the regulatory molecules involved in the process of reorganization. Towards this end, my laboratory identified a monoclonal antibody, mAb2A, which labels *Drosophila* nuclei in a striking cell cycle-specific pattern, identifying a nuclear meshwork at interphase which reorganizes into a spindle-like structure during

prophase and which co-localizes with the mitotic spindle at metaphase. Figure 1 depicts representative mAb2A staining compared with DNA and α-tubulin localization during different stages of mitosis. At interphase the mAb2A staining reveals a nuclear meshwork staining which overlaps with the Hoechst DNA labeling pattern, while tubulin is excluded from the nucleus at this stage, being localized in a cortical cap above and around the nucleus. In contrast, during metaphase the mAb2A staining co-localizes with the microtubule spindle fibers but not the asters. This spindle structure is observed through anaphase, but at telophase as the DNA begins to decondense, the mAb2A-labeled structures also decondense, being notably absent from the tubulin asters and mid-bodies.

At present there are two 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, could reflect the structural reorganization of a nuclear scaffold stained by mAb2A during the cell cycle. Since recent data from double and triple labelings demonstrate that the mAb2A-stained spindle-type structure observed in prophase precedes the establishment of the microtubule spindle apparatus and is formed at a time when tubulin is still excluded from the nucleus (Johansen, Johansen, and Baek, manuscript in preparation), we favor a model in which the mAb2A-stained nuclear meshwork itself reorganizes during the cell cycle. This meshwork forms a diffuse scaffolding at interphase overlapping with the DNA staining pattern, but reorients during prophase (Fig. 2) to establish a spindle-like scaffolding (the 2A spindle) which at this stage is distinct from the microtubules. At metaphase the 2A spindle co-localizes with the microtubule mitotic spindle, and thus it may provide a guide for assembly of the mitotic apparatus. Confocal microscopy confirms that the mAb2A-labeled structure forms a true spindle (Fig. 3); however, it also reveals filamentous staining at the metaphase-plate (Fig. 3, arrows) reminiscent of nuclear matrix core filaments resolved by resin-less EM imaging [Nickerson and Penman, 1992]. This nuclear cytoarchitectural reorganization revealed by mAb2A staining during the cell cycle suggests that some components of the nuclear matrix or some as-yet undefined nuclear component may be important for nuclear morphology and its changes during the cell cycle.

mAb2A Recognizes Two Distinct Antigens

We have determined that mAb2A recognizes two novel independent nuclear-specific antigens with similar distribution patterns [Walker et al., 1995]. For one, temporarily designated 2Ab3, the relatively short sequence in hand appears unique, while the other shows striking homology to serine-threonine kinases. The organization of this kinase homologue is highly unusual, consisting of two kinase-like catalytic domains bounded by a moderate (~15 kD) C-terminus and a substantial (~90 kD) N-terminal domain (Fig. 4). This double kinase domain organization is very rare, being found to date in one other serine-threonine kinase, the S6 kinase II [Jones et al., 1988], which consists of essentially only the two kinase-like domains. The mAb2A-identified antigen is organizationally much more similar to members of the Janus kinase (JAK) family, which also have large N-terminal domains before the tandem kinase-like domains followed by a shorter C-terminal tail [reviewed in Wilks and Harpur, 1994]; however the JAK kinases are tyrosine kinases. Thus, although there are significant homologies to S6 kinase II, this protein appears to define a totally new family of JAK-like serine-threonine kinases which we propose to name JIL-kinases (Johansen, Jin, Walker, Dong, Conley and Johansen, manuscript in preparation) and the kinase identified by mAb2A, JIL-1.

It is presently unclear what the relationship is between the two antigens recognized by mAb2A. Newly generated antibodies against each show similar staining patterns to the mAb2A staining pattern yet each identifies unique mRNA transcripts and proteins [Johansen and Johansen, 1994; Walker et al., 1995]. JIL-1 encodes a 5.3 kb mRNA consistent with a triplet of protein bands ranging from 170–180 kD detected on Western blots with anti-JIL-1 antibody, while the second identified locus encodes a 6.5 kb mRNA consistent with a 200 kD protein detected with anti-2Ab3 antibody.

The identification of a kinase which appears to co-localize with the nuclear matrix is intriguing. Mitosis is critically dependent on regulatory controls involving phosphorylation including release from cell cycle checkpoints [reviewed in Murray and Hunt, 1993], condensation of the chromosomes [Gurley et al., 1978; Matsumoto

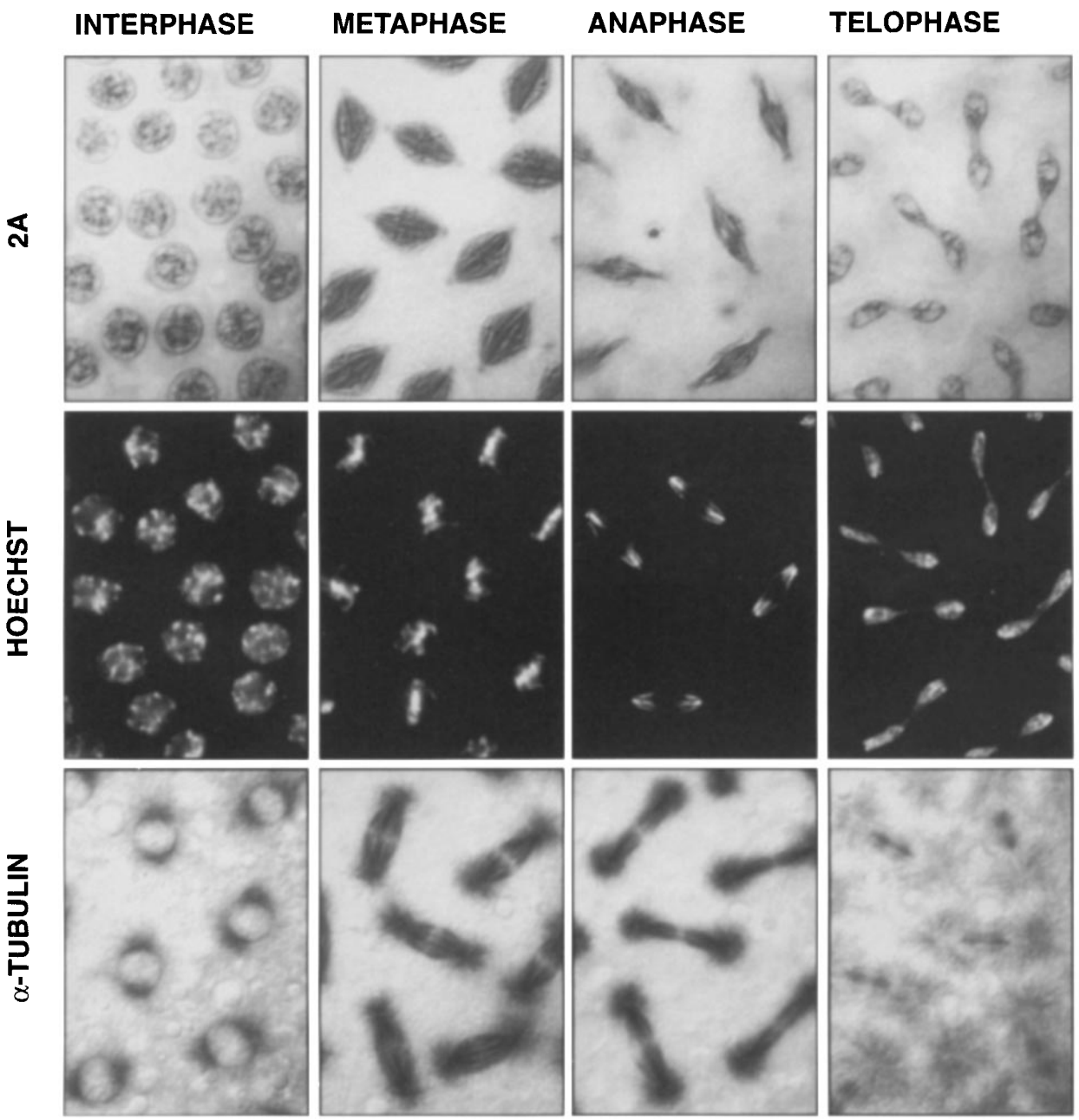

Fig. 1. Staining of staged *Drosophila* embryonic nuclei according to cell cycle phase using mAb2A, Hoechst, and α-tubulin antibody. The figure compares staining patterns of mAb2A (**top row**), DNA (**middle row**), and α-tubulin (**bottom row**) during interphase, metaphase, anaphase, and telophase. The mAb2A staining pattern demonstrates a dynamic redistribution pattern during mitosis. During interphase the mAb2A staining pattern overlaps with the Hoechst staining pattern for DNA, while at this stage α-tubulin is localized in a cortical cap above and around the nucleus. In contrast, during metaphase the mAb2A labeling appears to co-localize with α-tubulin on the microtubule spindle fibers but not on the asters, while the condensed chromosomes have congressed to the metaphase plate. This spindle structure remains labeled with mAb2A throughout anaphase but appears to redistribute during telophase, with the mAb2A-labeling pattern once again revealing a diffuse meshwork of staining overlapping with the Hoechst DNA staining. Panels represent typical staining patterns of syncytial blastoderm embryos fixed in Bouin's Fluid or PFAT (4% paraformaldehyde with 1 μM taxol), selected to represent matched cell cycle stages. The mAb2A and α-tubulin antibody (Sigma) were visualized using HRP-conjugated secondary antibodies.

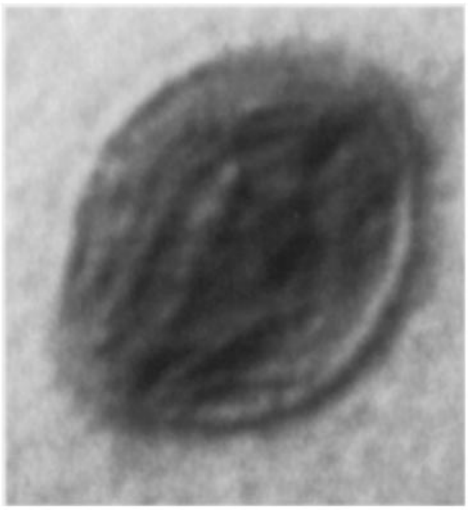

Fig. 2. The mAb2A-labeled spindle is well established by prometaphase. During prophase, the mAb2A-labeled meshwork begins to reorganize into a spindle-like structure which by prometaphase is clearly resolved. This figure indicates a prometaphase-stage nucleus labeled with mAb2A and visualized with an HRP-conjugated secondary antibody. At this stage, the microtubules are just beginning to assemble spindle fibers as the lamina begins to break down at the nuclear poles [Hiraoka et al., 1990].

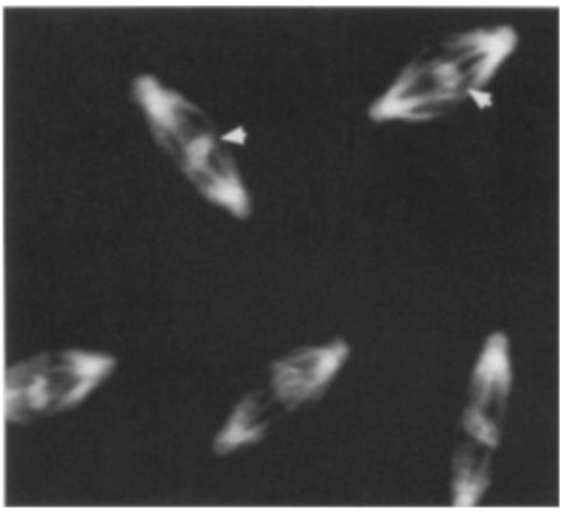

Fig. 3. Confocal microscopy of metaphase stage nuclei labeled using mAb2A reveals labeling of a true spindle structure. Metaphase stage nuclei were labeled with mAb2A and visualized using a TRITC-conjugated secondary antibody. The mAb2A-labeled structure is clearly resolved as a spindle. In addition, fibrous staining can be seen at the metaphase plate region (*arrows*).

et al., 1980], disassembly of the nuclear lamins during nuclear envelope breakdown [Gerace and Blobel, 1980], assembly of the mitotic spindle [Centonze and Borisy, 1990] as well as disassembly of the mitotic spindle [Dinsmore and Sloboda, 1988]. In particular, the formation of mitotic spindles can be blocked by injection of antibodies recognizing a phosphoepitope [Davis et al., 1989]. Mutation of predicted phosphorylation sites in NuMA result in both mislocalization of NuMA protein as well as disorganized, non-functional mitotic spindles [Compton and Luo, 1995]. These findings suggest that kinases are essential throughout the cell cycle and play a major role in the structural remodeling of the nucleus during cell division.

mAb2A Injections Into Living Embryos Perturb Normal Nuclear Division

The dynamic staining patterns observed with mAb2A suggests that the 2A antigens play an important role in nuclear division. In order to directly test this hypothesis, we performed antibody perturbation studies using mAb2A and control antibodies. The early *Drosophila* embryo is particularly advantageous for such studies: the cytoskeleton and mitotic spindles are large and easily visualized, thus facilitating structural analysis. The embryo undergoes 13 rapid and nearly synchronous nuclear divisions giving rise to about 6000 nuclei before cell boundaries form after 3 hours of development [Zalokar and Erk, 1976]. This syncytial organization of nuclei affords excellent accessibility for experimental perturbations using antibodies.

mAb2A ascites was injected into embryos 30 min post-laying and development allowed to proceed for 2.5 h before fixing and Hoechst staining. Embryos injected with mAb2A showed abnormal patterns of Hoechst staining as well as a striking reduction in the number of nuclei [Johansen, Johansen, and Baek, manuscript in preparation]. Control embryos injected with MOPC 104E ascites [Cappell; also an IgM-containing ascites] and similarly treated were indistinguishable from wild-type, showing no perturbation effects. These data show that blocking one or both of the mAb2A antigens interferes with mitosis, thus indicating an essential role for the antigen(s) in cell division. The apparent disintegration of nuclear structure seen in severely perturbed nuclei is consistent with the antigens' association with the nuclear matrix or an as-yet undescribed nuclear scaffold, as observed in mAb2A stainings of embryos (Figs. 1 and 2) and is consistent with the hypothesis that the 2A antigen(s) are important for nuclear structural integrity.

Fig. 4. Schematic diagram of JIL-1. Sequencing of partial cDNAs encoding one of the antigens recognized by mAb2A reveals an unusual tandem kinase domain. Each of the kinase domains shows homology to serine-threonine kinases, and this double kinase region is followed by an approximately 15 kD C-terminal tail. Based on estimation of protein size by Northern and Western blot analysis, we predict an approximately 90 kD N-terminus (*hatched region*).

Drosophila as a Model System for Studying Mitosis

Our understanding of the cell cycle and mitosis has increased significantly in the last several years due to cross-disciplinary approaches combining molecular, cell biological, and genetic techniques [reviewed in Murray and Hunt, 1993; Norbury and Nurse, 1992]. One particularly striking outcome of these studies has been the demonstration of the extraordinary degree of structural and functional conservation across evolution of the signaling molecules involved in the regulation of cell division, most dramatically demonstrated by the fact that molecules found in humans can rescue cell cycle mutants in yeast [Lee and Nurse, 1987]. *Drosophila* has proven to be an excellent model system for the analysis of signal transduction pathways because genetic screens can be done to isolate other components in a pathway (suppresser and enhancer screens) when one component of the pathway has been identified. Many tools are also available to assay phenotypic effects (antibodies, enhancer-trap lines, tissue- and cell-specific mutant markers), and gene function can be readily analyzed using molecular and cell biological techniques. Both the R7 photoreceptor cell pathway [Hafen et al., 1987] and embryonic terminal differentiation pathway [Ambrosio et al., 1989] are examples where these advantages in *Drosophila* have allowed identification of a number of additional members involved, and where regulatory mechanisms for differentiation control are being analyzed on the molecular level.

Using a monoclonal antibody which specifically stains a nuclear meshwork which reorganizes during the cell cycle in *Drosophila,* we have initiated a study taking advantage of the *Drosophila* system to more closely analyze this structural reorganization, to clone and characterize the antigens recognized by this antibody, and to isolate other interacting proteins in order to gain insight into the regulation of this process. Our studies indicate that the mAb2A-labeled nuclear meshwork reorganizes from prophase through prometaphase to form a novel spindle-like scaffold. This scaffold precedes the formation of the microtubule spindle apparatus but is co-localized with it at metaphase, suggesting it may serve as a guide for microtubule spindle assembly (Johansen, Johansen, and Baek, manuscript in preparation). The mAb2A recognizes two distinct antigens, both of which show similar stage-specific nuclear distribution patterns. We are currently purifying antibody probes specific to each for injection into syncytial blastoderm embryos in order to assay individually the functional role of each of the two mAb2A antigens. This may allow us to separate some aspects of the 2A antigens' functions.

The identification of a tandem kinase domain in one of these antigens, JIL-1, coupled with its cell-cycle specific distribution pattern in the nucleus, suggests that JIL-1 may be involved in signal transduction events regulating and/or responding to mitotic events. Future identification of mutants in JIL-1 in combination with perturbation analysis studies currently underway will allow us to determine the function of JIL-1 and to identify the signal transduction pathway(s) in which it operates. Our current hypothesis is that JIL-1 kinase activity may trigger the stage-specific remodeling of the scaffold, directing its reorientation to create a spindle-like scaffold which could serve as a guide for establishment of the mitotic apparatus by stabilizing spindle microtubules and serving as a scaffold for interacting motor proteins. JIL-1-stimulated remodeling may also promote establishment of the filamentous network associated with the metaphase chromosomes which may play a role in orienting and partitioning nuclear components. In our model we envision a more active role of the nuclear matrix in providing a guide throughout the cell cycle, ensuring the proper partitioning of nuclear contents and regulating establishment of appropriate functional domains. Since the signaling pathways used in *Drosophila* appear to be highly conserved in higher eukaryotic systems, there is great promise that information gained on JIL-1's role and p2Ab3's possible role in mitotic regulation will

yield insight into mitotic regulatory control mechanisms applicable in other systems, including human.

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