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The Nucleocytoplasmic Continuum: Pushing the (Nuclear) Envelope

The nucleus has been recognized as a quintessential feature of the cell for well over a hundred years, but throughout the century subsequent to Friedrich Miescher's 1869 discovery of "nuclein," or nucleic acid, the questions that most intrigued biologists concerned the nature of the macromolecules that the nucleus contains. Certainly the attention given to the nucleic acids was warranted. After all, it had been established by the turn of the century that the nucleus determined inheritance, and much of the chromosome behavior that we now know as mitosis and meiosis had been described. But the nucleus as a cellular organelle, defined primarily by an "envelope" with holes in it, hardly seemed spectacular. Indeed, its very existence was relegated to a period of cellular metabolism known as *interphase*, which microscopists had to tolerate until the nuclear envelope would finally disappear and the chromosomes would resume the more fascinating aspects of mitosis.

In the last quarter century, as additional details of gene structure, regulation, and expression have been elaborated, new questions have arisen so as to shift attention back to the interphase nucleus. A major conceptual challenge, for example, has been to understand the mechanisms whereby a genome's worth of DNA, which in some instances can be extended to the order of a meter in length, is condensed into a nucleus that measures on the order of a few micrometers in diameter. Beyond the specific association of genomic DNA with histones so as to produce the 10- and 30-nm fibers first described in the 1970s, it is still not fully clear how nuclear DNA can be made so compact. On top of the problem of DNA condensation per se are the equally formidable challenges of understanding (1) how

thousands of gene sequences and gene regulatory elements can be specifically accessed within the tightly packed interphase nucleus and (2) how macromolecules can be specifically targeted for entrance into and exit from the conceptual jumble of nuclear stuffing. The interphase nucleus, no longer seen as a mere receptacle that sequesters the hereditary material away from the cytoplasmic milieu, is now pursued for the ways that it imposes order, and yet allows flexibility, within the operating genome.

The current view of the nucleus endows the organelle with multiple functionalities, the structural bases of which remain largely obscure. It is clear, however, that the nuclear envelope is an intimate participant in establishing functional domains, and itself functions in the dynamics of gene expression. Some of the greatest advances in this latter regard revolve around the "holes" that were suspected a priori and found in early electron micrographs of the nuclear membrane (see Bonner, 1965). The nuclear pore complexes (NPCs) that have been described since the 1980s represent some of the most elaborate macromolecular complexes in the cell and consist of over 100 different proteins that together result in a mass of ~125 MD. The aqueous channel at the center of each NPC is believed to allow for the diffusion of organic solutes as well as its more prodigious feat of actively transporting diverse species of RNA, DNA, proteins, and nucleoprotein particles (Davis, 1995). The majority of the protein components (nucleoporins) of the NPC remain uncharacterized, but it is clear that NPCs from diverse organisms, including plants (Heese-Peck and Raikhel, 1998), are well conserved.

With the advent of fluorescent in situ

hybridization (FISH; chromosome painting), new insight into the topology of the chromatin within the interphase nucleus has been made. Indeed, it is now even possible to discuss "interphase chromosomes," a term that would have been dismissed as internally contradictory a little over a decade ago. The use of FISH has made it apparent that decondensed chromosomes in the interphase nucleus are not all tangled up together but rather assume non-overlapping configurations, perhaps as a consequence of specific DNA-nuclear envelope interactions (see Franklin and Cande, 1999).

But remove the chromatin from isolated nuclei by nuclease treatment, and extract soluble proteins by high salt or detergent, and what's left? The insoluble material left over after such treatments is generally referred to as the "nuclear matrix" (Berezney and Coffey, 1974) and is regarded, by analogy to the cytoskeleton of the cytoplasm, as a protein meshwork that organizes the nuclear contents and facilitates nucleocytoplasmic movement of macromolecules. Indeed, specific AT-rich sequences of DNA that remain associated with the interphase nuclear matrix subsequent to DNase treatment have been identified in yeast, mammals, and plants. These residual DNA sequences, referred to as MARs (for matrix-associated regions) occur in noncoding regions and often flank genes (Gasser and Laemmli, 1987). They are thus envisaged to tether genetic loci, manifested as loops emanating from the nuclear matrix, to a nuclear scaffold, where these are made accessible to the transcriptional enzymatic and regulatory machinery. In this way, the nuclear matrix can be seen to take on the fundamental role of establishing functional domains within the nucleus.

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Although the *in vivo* existence of the nuclear matrix was initially regarded with some skepticism, discrete "islands" or "speckles" of gene-processing complexes, visualized by immunofluorescence microscopy and other methods, suggest that nuclear functions such as transcription, RNA splicing, and initiation of DNA replication are localized into nuclear domains (see, e.g., Wei et al., 1998). Mammalian transcription enhancer elements and origins of DNA replication have been found to collaborate with flanking MARs (Cossons et al., 1997; Jenuwein et al., 1997). For this reason, the identification of nuclear matrix proteins that bind to MARs so as to orient DNA sequences (e.g., enhancer elements and promoters) with respect to one another as well as to proteins (e.g., transcription factors) is crucial to a full understanding of the orchestration of nuclear metabolism.

In animal cells, the most thoroughly studied protein components of the nuclear matrix are the three classes of nuclear lamins (A, B, and C) that form the laminar network of filaments directly beneath the nuclear envelope. Lamin B is anchored to the inner nuclear membrane through a farnesylated cysteine residue (Farnsworth et al., 1989) and interacts with lamins A and C. Upon copolymerization, moreover, the lamins manifest MAR binding activity (Luderus et al., 1994). Many other proteins with MAR binding activity have been isolated, but their relationships to the nuclear matrix remain largely unclear.

On pages 1117–1128 of this issue, Gindullis and Meier describe their characterization of a plant protein, intimately related to the nuclear matrix, with MAR binding activity. Some of the characteristics of the protein, known as MFP1 (for MAR binding filament-like protein 1), have been reported previously (Meier et al., 1996). In addition to the MAR affinity for which it was named, MFP1 was implicated on the basis of a predicted α -helical domain similar to that of cytoplasmic filament

proteins as a structural element of the nuclear matrix. MFP1 was additionally confirmed as a phosphorylatable substrate of a nuclear matrix-associated kinase; significantly, the mitotic depolymerization of the nuclear lamina is effected upon phosphorylation of serine residues. For these reasons alone, MFP1 is an intriguing candidate for further studies into the nature of the architecture within the plant cell nucleus.

In their present report, Gindullis and Meier painstakingly establish the localization of MFP1, through both immunocytochemical methodology as well as analysis of the expression of GFP fusion constructs that are elaborated as "speckles," to the nuclear rim of isolated nuclei and nuclear matrix. They have also confirmed through deletion analysis that the hydrophobic N-terminal domain of MFP1 specifically localizes the protein to the nuclear rim, probably by functioning as a transmembrane anchor. Beyond establishing interactions of the N terminus with nuclear membrane, however, the authors argue that additional, nonmembrane, interactions connect MFP1 to the nuclear matrix. This exciting conclusion, arguing that MFP1 is organized by protein–protein and protein–MAR interactions into a matrix, arises from the persistence of MFP1 at the nuclear rim despite both the significant deletion of the N terminus and detergent treatment that removes significant membrane material.

Perhaps most provocatively, the authors investigate whether additional structural components of the cell could be found to interact with MFP1 in its proposed role as a nuclear matrix component. Their approach may seem counterintuitive, inasmuch as the interaction that they seek—and find—lies outside the nucleus. Specifically, because the outer nuclear surface of plant cells appears to function as a microtubule organizing center (MTOC) and in fact seems to replace the function of the centrosomes of animal cells (re-

viewed in Franklin and Cande, 1999), the authors investigate whether the plant nuclear matrix, on antigenic grounds, manifests itself as an MTOC. Indeed, their rather spectacular result is that mAbC6C, a monoclonal antibody raised against calf thymus centrosomes, reacts with the surface of nuclear matrix preparations to yield a speckling pattern reminiscent of MFP1. Unlike MFP1, the mAbC6C antigen does not penetrate beneath the surface so as to participate directly in the intranuclear architecture. Interestingly, however, to the extent that the nuclear matrix has been envisioned to promote macromolecular movement by analogy to the cytoskeleton, the authors' results challenge the traditional separation between nuclear and cytoplasmic function. In any event, the case being made for the plant nuclear matrix and the function of MFP1 therein is strengthened by Gindullis and Meier, and their experimental approaches will likely yield additional insights into the nuclear matrix in the near future.

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Trends in Plant Cell Cycle Research

Plant growth can be considered as the sum of cell proliferation in the meristems and the subsequent elongation of cells. The continuous proliferative capacity of plant cells is crucial for the production of new organs and thus has a significant impact on plant architecture. The questions to be addressed are what controls the entry, maintenance, and exit of the cell cycle? What are the molecular components of the plant cell cycle machinery, and which roles do they play in differentiated cells and during development? How does the cell cycle machinery interact with the cytoskeleton? These areas of research have experienced considerable progress in recent years (Mironov et al., 1999), and the molecular tools required to approach them in a developmental context are gradually becoming available.

A workshop titled "Cell Cycle Regulation and Cytoskeleton" was recently sponsored by the Instituto Juan March de Estudios e Investigaciones in Madrid (Spain) on March 22–24, 1999. This meeting provided a timely platform for discussions of current topics in the regulation of the cell cycle and cytoskeleton in plants. The present meeting

report reflects some of the meeting highlights.

CYCLIN-DEPENDENT KINASES

As in other eukaryotes, cyclin-dependent kinases (CDKs) are central key regulators of the plant cell cycle (Doonan and Fobert, 1997). Extensive work in *Arabidopsis*, tobacco, and alfalfa demonstrates that plants possess at least four different classes of CDKs. Best characterized are the CDKs containing the hallmark PSTAIRE sequence, which play a role in both the G₁-to-S and G₂-to-M transitions. An additional class of CDKs, unique to plants, is defined by a PPTALRE sequence and protein and activity levels that are highest at late G₂ and M phases. As postulated by several meeting participants, this group of CDKs might regulate plant-specific aspects of mitosis (Jim Murray, Institute of Biotechnology, University of Cambridge, Cambridge, UK; Dènes Dudits, Institute of Plant Biology, Szeged, Hungary; Dirk Inzé, Flanders Interuniversity for Bio-

technology, Gent, Belgium). Dirk Inzé further showed that transgenic plants that overexpress a dominant negative mutant of the PPTALRE-containing CDK from *Arabidopsis* tend to develop cells with 4C content, indicating that the kinase plays a pivotal role in the G₂-to-M transition.

CYCLINS

The multiplicity of cyclins upon which plant CDKs depend generally exceeds that of animal cells (Renaudin et al., 1996). At least four different classes of plant cyclins (A, B, D, and H) can be found. Jim Murray and Dirk Inzé presented evidence that D-type cyclins control re-entry into the cell cycle and are important integrators of developmental signals into the cell cycle. As an example of such integration, two *Arabidopsis* D-type cyclins (CycD2 and CycD4) are induced by sucrose, whereas CycD3 is specifically induced by cytokinins. John Doonan (Department of Cell Biology, John Innes Institute, Norwich, UK) showed that the two D3-type