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Towards an Understanding of Nuclear Morphogenesis

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Abstract In the age of "virtual reality," the imperfect microscopic silhouettes of cells and organelles are gradually being replaced by calligraphic computer drawings. In this context, textbooks and introductory slides often depict the cell nucleus as a smooth-shaped, featureless object. However, in reality, the nuclei of different cells possess distinct sizes and morphological features which develop in a programmed fashion as each cell differentiates. To dissect this complex morphogenetic process, we need to identify the basic elements that determine nuclear architecture and the regulatory factors involved. Recently, clues about the identity of these components have been obtained both by systematic analysis and by serendipity. This review summarizes a few recent findings and ideas that may serve as a first forum for future discussions and, I hope, for further work on this topic. © 1994 Wiley-Liss, Inc.

Key words: differentiation, nuclear lamina, intermediate filaments, nuclear matrix

The various cell types of multicellular organisms differ in several morphological features. This phenotypic diversity reflects a functional diversity, i.e., the fact that each cell contains a specialized machinery which allows it to carry out specific physiological tasks. On the other hand, eukaryotic cells resemble each other because they all share a set of conserved structures and "housekeeping" organelles. A typical example of such a "generic" organelle is the cell nucleus.

Considering the central role of the nucleus in cellular physiology, one would expect the architecture of this organelle to be the same in all cells of the body. However, it is widely known that the shape, size, and intracellular location of the nucleus all vary markedly depending on the cell type and the state of differentiation. For example, whereas the nuclei of cortical CNS neurons are round and centrally located, the nuclei of skeletal muscle fibers are elongated and peripherally disposed. In other instances, the nucleus is lobular and segmented (polymorph leukocytes), bullet like (spermatocytes), or eccentric (adipocytes, plasma cells) (for a few illustrative examples see Fig. 1). Furthermore, in contrast to the idealized image of a smooth-shaped nucleus, the nuclear membrane often contains folds or indentations extending deep into the nuclear interior (Sertoli cells, epithelial cells of the intestine, promegakaryocytes). Finally, the nucleus may be functionally polarized. This can be inferred from recent experiments showing that certain mRNA transcripts produced in blastoderm embryos of *Drosophila melanogaster* are asymmetrically distributed and probably exit the nucleus either apically or basolaterally [Davis and Ish-Horowicz, 1991].

Realizing the poikilomorphism of the cell nucleus, it becomes important to discuss the spectrum of factors that determine nuclear architecture and the postmitotic mechanisms that may change this architecture. Due to space limitations, I will not review here a related topic, i.e., the dynamics of nuclear assembly and disassembly during cell division. It suffices to say that many aspects of regulation of nuclear envelope disassembly and reassembly during mitosis may be similar to the mechanisms used to change nuclear structure during development and differentiation.

STRUCTURAL ELEMENTS AFFECTING NUCLEAR ARCHITECTURE

The Three-Dimensional Structure of the Genome

Under in vivo conditions, genomic DNA is packaged in nucleosomes and is further organized into a larger supramolecular formation, the chromatin network. There are several chromatin "folding states": the 11 nm "beads on a string" fiber, the helical 30 nm fiber, and the

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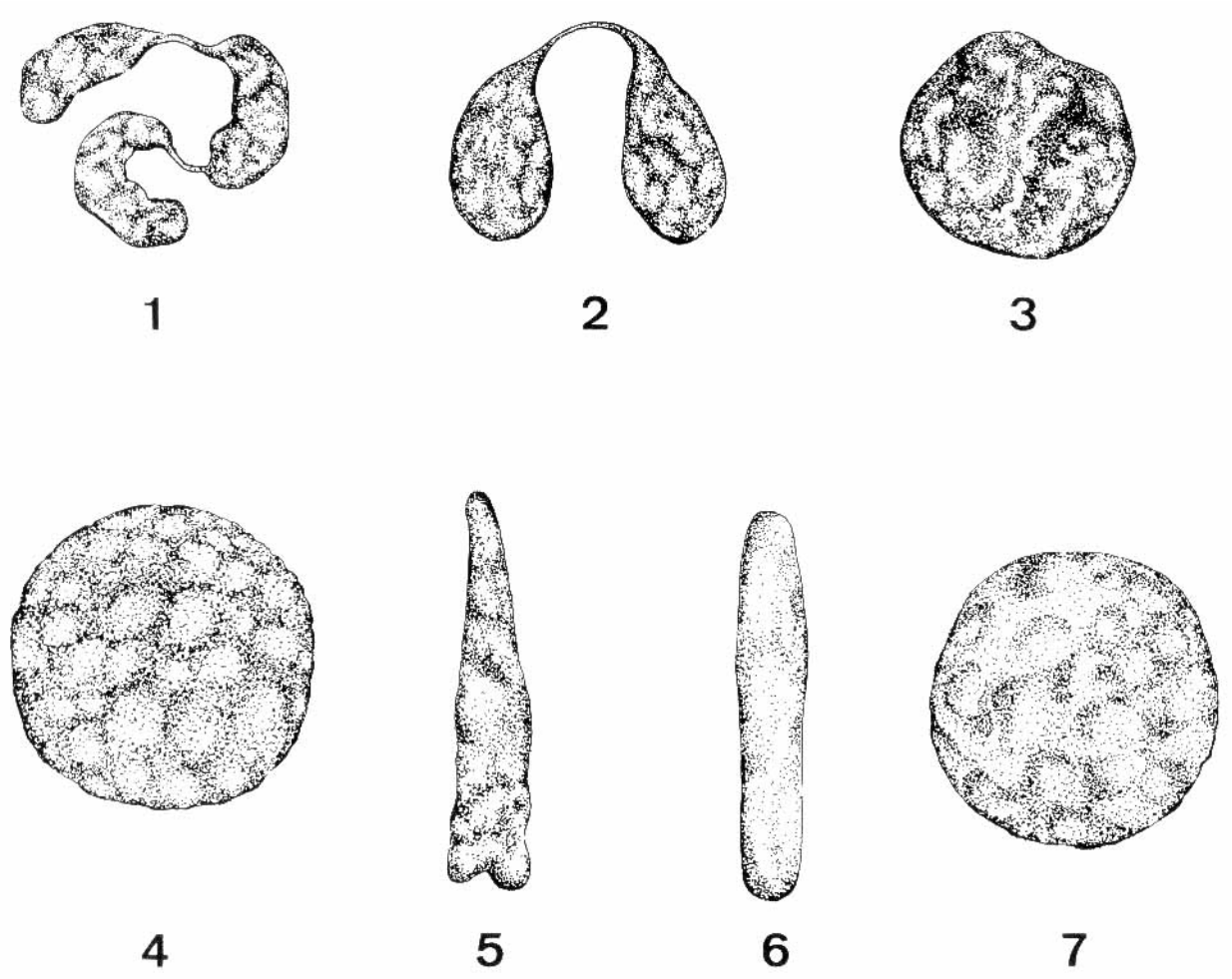

Fig. 1. Schematic representation of some human cell nuclei. **1:** Nucleus of a neutrophil leukocyte; **2:** nucleus of an eosinophil leukocyte; **3:** nucleus of a lymphocyte; **4:** nucleus of a CNS neuron; **5:** nucleus of a spermatocyte; **6:** nucleus of a skeletal mucle fiber; **7:** nucleus of a hepatocyte. Note the variations in shape and surface morphology. The sizes of the different nuclei are not to scale.

postulated chromatin "loops," segments of 50–100 kb of DNA whose base is thought to attach to the nuclear matrix [reviewed in Earnshaw, 1991]. In addition, chromatin comprises alternating "compacted" (heterochromatic) and "expanded" (euchromatic) macrodomains. In this spatial framework, the genome has been postulated to have a distinct three-dimensional (3-D) structure, similar to the 3-D structure of a folded protein [Blobel, 1985]. The 3-D structure of the genome may be peculiar to the differentiation state of each cell because the pattern of euchromatic and heterochromatic regions, as well as the arrangement of chromatin attachment sites in the nuclear matrix, are expected to vary during ontogeny. In addition, the 3-D structure of the genome may influence the global morphology of the nucleus, for each cell will have to accommodate the nuclear content into a membranous compartment which (at least roughly) matches the "hills" and "valleys" of the chromatin network. Alternatively, the nuclear envelope may modulate the 3-D structure of the genome by providing specific attachment sites for chromatin (see Adaptability and Flexibility of Nuclear Structures).

The Nuclear Matrix

The nuclear matrix represents a "ghost" of the nucleus obtained after extraction of cells with salt, detergents, or chaotropic agents. Whether this "residue" represents the underlying infrastructure of the nucleus or is an in vitro artefact has been debated for over a decade. Despite the controversy, there are two sets of results concerning the nuclear matrix that are

worthy to discuss. First, the matrix seems to contain filamentous elements identifiable by whole-mount electron microscopy [e.g., Fey et al., 1984]. Some of these nucleoplasmic fibers have a diameter of 10 nm and an axial periodicity of 23 nm [Jackson and Cook, 1988], i.e., precisely the ultrastructural features of the intermediate-sized filaments (IFs). The possibility that the nucleoplasmic filaments represent aggregated ribonucleoprotein particles does not seem likely because the latter are known to possess quite different characteristics (a diameter of 18 nm and an axial repeat of 60 nm). In analogy to the cytoplasmic IFs, the nucleoplasmic 10 nm filaments may mechanically support the nucleus and contribute in the peculiarities of nuclear architecture. Second, the nucleoplasmic filaments may be involved in cytoplasmic–nucleoplasmic transport. This is suggested by recent in situ studies on nonextracted cells showing that a protein that shuttles between the cytoplasm and the nucleolus is aligned along "tracks" [Meier and Blobel, 1992], whereas newly synthesized RNA, presumably on its way to the nuclear pores, can also be localized in linear arrays at specific regions of the nuclear interior [Xing et al., 1993]. However, since in both cases only the passenger and not the actual "tracks" have been visualized, these findings can also be explained by "queuing" of the transported material in front of the sites of entry and exit from the nucleus.

The protein subunits of the nucleoplasmic filaments are not known. Nevertheless, new information now suggests that at least a subset of these structures may be composed of a nuclear matrix protein termed NuMa [Yang et al., 1992; Compton et al., 1992 and references therein]. NuMa is a 236 kDa polypeptide which may polymerize into filaments because it possesses a long coiled-coil domain, similar to the "rod" domains of other fibrous proteins. It is found exclusively in the nucleoplasm during interphase but during mitosis associates with the centrosomes and concentrates at the poles around the telophase chromosomes. Microinjection of anti-NuMa antibodies and transfection of cells with truncated NuMa constructs lead to the formation of many micronuclei instead of two daughter nuclei, implying that this protein may somehow tether the chromosomes together at the end of mitosis [Compton and Cleveland, 1993; Kallajoki et al., 1993]. There has also been some immunohistochemical evidence supporting the idea that the nucleoplasm may contain lamins, the building blocks of the fibrous nuclear lamina, or lamin-related proteins [Bridger et al., 1993]. Finally, it has long been known that the nucleus of some cells contains actin [e.g., Clark and Merriam, 1977] and isoforms of the actin-binding protein 4.1 [Correas, 1991]. However, the existence of an organized system of intranuclear actin microfilaments has not been documented yet.

The Nuclear Envelope and Associated Structures

As mentioned above, one may consider the nuclear envelope as a "mold" of the chromatin network. This is consistent with what has actually been observed in situ. For example, high resolution microscopy on intact nuclei from *Drosophila melanogaster* has shown that the nuclear lamina and a large fraction ($\sim 65\%$) of peripheral chromatin are coaligned along the nuclear periphery [Paddy et al., 1990]. This striking coalignment brings about the possibility of a direct interaction between the nuclear lamina and the chromatin network. The nuclear lamina represents a system of intermediate filaments (IFs) interposed between the chromatin and the inner nuclear membrane [Aebi et al., 1986]. It is composed of protein subunits, the lamins, which belong to two general classes: the type A and the type B lamins. The lamins are thought to interact with the inner nuclear membrane by binding to integral membrane proteins, such as the LAPs (lamina associated polypeptides) [see Foisner and Gerace, 1993] and the p58 (also known as the "lamin B receptor") [Worman et al., 1988a; Simos and Georgatos, 1992]. Both types of lamins are able to bind specifically to chromatin fragments in vitro [Glass et al., 1993 and references therein]. Based on this, it has been proposed that the nuclear lamins physically interact with a proteinaceous component of the chromatin.

The interactions between the nuclear lamins and chromatin may be pivotal for function. Yet, these associations do not seem to explain the extensive coalignment of the two structures because high-resolution optical microscopy shows that the space intervening between the nuclear lamina and the chromatin is quite substantial (in the order of 200 nm) and that in only a few foci (2–3/chromosome) is the lamina near enough to the chromatin to allow for direct binding [Paddy et al., 1990]. If the nuclear lamins are not the principal factors that couple the chromatin to the nuclear envelope, what then

are the elements responsible for the coalignment of the nuclear lamina and the chromatin? New biochemical and morphological studies have shed some light on this problem. First, it has been shown that integral membrane proteins of the nuclear envelope (such as the LAPs) may directly associate with chromatin [Foisner and Gerace, 1993]. The LAPs, being bifunctional, have the capacity to serve as "adaptors" which mediate the coupling of the chromatin to the nuclear lamina. Second, at least in some cell types, apart from the nuclear lamina proper there is another fibrous network, termed the nuclear envelope lattice [Goldberg and Allen, 1992]. This network appears to be more closely associated with the pore complex and less with the fibrous lamina but it is still possible that it may act as an interface between the lamina and the chromatin.

The Cytoplasmic Intermediate Filaments

The cytoplasmic IFs represent a major cytoskeletal system closely associated with the cell nucleus. IFs have often been described as a fibrous basket which "nests" the nucleus without being in physical contact with it. Alternatively, other authors have indicated that IFs organize as a radial network directly attached to the nuclear surface. While the debate continues, the latter scenario has received more experimental support than the former. I will cite below some examples that are worthy of consideration.

First, desmin IFs, associated with the Z disks of muscle cells, are known to approach the nuclear membrane at focal points. At these sites, the surface of the nucleus appears to be "wrinkled" or "elevated," as if the filaments were "pinching" on the nuclear envelope. The linkage between desmin IFs and the nuclear envelope seems to be tight because the length of the nucleus and the surface morphology of the nuclear envelope changes as the length of the sarcomere is altered during contraction and relaxation [reviewed in Tokuyasu et al., 1985]. These data not only suggest a physical interaction between the filaments and the surface of the nucleus but also indicate that this interaction is regulated. The same point is supported by a second example involving MPC-11 mouse plasmacytoma cells deficient in cytoplasmic IFs. Such cells have been found to possess an anomalous nuclear surface and a very fragile nuclear envelope [Wang and Traub, 1991]. Along the same lines, keratinocytes expressing mutated keratins and possessing easily fragmenting IFs have also been found to have characteristic nuclear abnormalities [reviewed in Fuchs and Coulombe, 1992]. Finally, recent studies with mitotic cells raise the tantalizing possibility that postmitotic nuclear reassembly may be dependent on cytoplasmic IFs. This idea is supported by microinjection experiments showing that antivimentin antibodies, when introduced into synchronized mitotic cells, induce a transient arrest in the M phase or polylobulation of the daughter nuclei [Kouklis et al., 1993]. Complementing these findings, it has been recently observed that lamin B–containing vesicles, probably derived from the fragmentation of the nuclear membrane during mitosis, are docked on vimentin IFs [Maison et al., 1993].

The simplest way to explain these data is to assume that, at some level, the cytoplasmic and nucleoplasmic filament networks are integrated into a functional unit. A paradigm which graphically depicts the potential for transmembrane interactions between intranuclear and cytoplasmic filaments comes from in situ studies on the distribution of lamin A and vimentin IFs in human promyelocytic leukemia (HL-60) cells [Collard et al., 1992]. Undifferentiated HL-60 cells do not contain vimentin IFs and possess a "cap" of lamin A, asymmetrically disposed on the nucleoplasmic side of the nuclear envelope. However, upon treatment with phorbol esters, vimentin synthesis is induced and the protein appears first at a focal region near the nucleus corresponding to the lamin A "cap." With time, the two "caps," one on the nucleoplasmic and one on the cytoplasmic side, start to spread in an apparently coordinated fashion.

Obviously, a direct contact between cytoplasmic IFs and nucleoplasmic lamin or NuMa filaments would not be possible because of the double nuclear membrane barrier. Yet, there are other alternatives which could be utilized to establish a transmembrane communication. For example, it is known that cytoplasmic IFs often connect to the nuclear pores via thin filamentous elements [Carmo-Fonseca et al., 1987]. It is also plausible to suggest that the two filament systems communicate through long-range interactions mediated by integral nuclear membrane proteins across the perinuclear space. The perinuclear cisterna represents the compartment enclosed between the inner and the outer nuclear membrane, has a width of 200–500 Å, and sometimes contains electron-dense granular mate-

rial. This space is equivalent but not identical to the lumen of the ER (endoplasmic reticulum), because it contains (in addition to ER proteins) the lumenal segments of inner nuclear membrane and pore-associated proteins. Conceivably, interactions between the cytoplasmic IFs and integral or peripheral proteins of the outer nuclear membrane may be relayed to the lumenal part of such inner membrane and pore-associated proteins, affecting other interactions taking place on the nucleoplasmic side.

ADAPTABILITY AND FLEXIBILITY OF NUCLEAR STRUCTURES

Differential Expression of Karyoskeletal Proteins During Development

Through a variety of studies it has been established that the nuclear lamins and their partners are differentially expressed during development. Thus, the A-type lamins appear relatively late, after the various cells have reached their differentiated state, whereas lamin B is constitutively produced in all stages of development [Rober et al., 1989]. Furthermore, the ratio of type A to type B lamins and the ratio of lamin B to p58 (the "lamin B receptor") vary depending on the cell type [Worman et al., 1988b; Bailer et al., 1991]. It is apparent that lamin heterogeneity may influence nuclear architecture. A good example of this has been provided by recent studies on mouse spermatocytes. These cells have a hook-like nucleus and express an isoform of lamin B termed lamin B3. It turns out that transfection of somatic cells with lamin B3–coding constructs causes a change in nuclear shape and converts the nucleus from spherical to hook shaped [Furukawa and Hotta, 1993]. This is perhaps the best evidence that the nuclear lamins can (directly or indirectly) cause changes in the 3-D structure of the genome.

In Situ Remodeling and Local Modification of Nuclear Components

A key to understanding nuclear morphogenesis is the elucidation of nuclear envelope and chromatin dynamics. The main components of the nucleoskeleton and the cytoskeleton, as well as the main components of chromatin (e.g., histones), are continuously modified during interphase and mitosis. So far, more emphasis has been given to mitotic modifications (principally cdc2-mediated phosphorylation) because these alterations mediate a dramatic and easily observable breakdown of interphase structures. However, interphase phosphorylation may be as important for the structural flexibility and the adaptability of nuclear structures to a changing nucleoplasmic or cytoplasmic milieu. For example, an increase in the size of the nucleus during interphase may require local relaxation of the lamina meshwork or/and accelerated incorporation of lamin subunits into the polymer. This may be achieved by local phosphorylation of the nuclear constituents by nuclear envelope kinases. Two such enzymes have been detected so far in vertebrates: one is a kinase associated with the nuclear envelope of Ehrlich ascites tumor cells which modifies the lamins and a 52 kDa nuclear envelope protein [Dessev et al., 1988]; the other is a specific kinase strongly associated with the inner nuclear membrane protein p58 in avian red blood cells [Simos and Georgatos, 1992]. The p58 kinase phosphorylates p58 in vivo, but does not modify the lamins or the histones. Interestingly, p58 phosphorylation, which is essential for lamin binding in vitro, seems to be inducible by β-adrenergic agents. This suggests that the nuclear lamina–nuclear membrane interactions could be modulated by environmental cues [Appelbaum et al., 1990]. Apart from these two kinases, there is evidence that the nuclear envelope contains a nucleotide triphosphatase activated by poly (A)–containing RNA [e.g., Bernd et al., 1982; Agutter et al., 1977]. Although this enzyme is thought to be involved in cytoplasmic–nucleoplasmic transport, it is conceivable that it may also participate in the local remodeling of the nuclear envelope.

From a theoretical viewpoint, the most "modulatable" feature of the nuclear envelope would be the meshing of the nuclear lamina network and the nuclear envelope lattice. Following the example of actin-binding proteins, one could imagine that the meshworks of intranuclear filaments could be dramatically altered by proteins which cross-link lamin filaments in a Ca^{2+} or phosphorylation-dependent fashion. Such lamin-bundling proteins have not yet been characterized; however, the existence of a very thick lamina in certain cell types [Hoeger et al., 1991] suggests that factors with an ability to laterally link lamin filaments may exist.

Finally, the lateral aggregation of lamin-binding proteins (LAPs, p58) and the formation of oligomeric clusters at the level of the inner nuclear membrane could also affect the degree

of crosslinking of lamin filaments. There is already some evidence supporting the idea that the first transmembrane domain of p58 may bind to p58 itself [Smith and Blobel, 1993]. Thus, polyvalent lamin-binding complexes may serve as nucleation centers from which new filaments could be initiated depending on physiological needs and the phase of the cell cycle.

"Relays" and "Hinges"

Another parameter that may be important in nuclear envelope and chromatin dynamics is the modulation (i.e., masking and unmasking) of potential interaction sites during the cell cycle and during development. From a large number of observations it seems reasonable to conclude that the potential for a physical interaction between two cellular components is not always determined by the chemical affinity for each other but also by their spatial proximity and the accessibility of the corresponding binding sites. Thus, chromatin may or may not interact with the nucleoskeleton depending on whether or not the corresponding interacting sites are available or occupied. This will depend, in turn, on a number of parameters including auxiliary factors and post-translational modifications that may "open" or "censor" potential binding sites. A prime example of this type of regulation is provided by the effects of acetylation on histone function. It has recently become known that H4 histone modified at Lys 16 is associated with transcriptionally hyperactive chromatin, whereas underacetylated H4 is primarily localized in heterochromatin. Acetylation has been proposed to "open" sites on histones and facilitate binding of other non histone proteins that regulate chromatin function [reviewed in Turner, 1993].

Most membrane–cytoskeleton contacts involve a multiplicity of interactions between macromolecular complexes arranged near the membrane. For example, the p58 protein forms a multimeric complex which includes the nuclear lamins, the p58 kinase, another membrane protein called p18, and a peripheral protein termed p34 [Simos and Georgatos, 1992]. It is possible that this "junctional unit" contains all the elements necessary for modulating the lamin–membrane interactions. For example, binding of lamin B to p58 may be regulated by phosphorylation of the latter by the complex-associated kinase. The p18 and p34 proteins may also influence (positively or negatively) the lamin–p58 interaction or bind lamins directly.

POTENTIAL PATHWAYS OF NUCLEAR MORPHOGENESIS

We could distinguish three potential pathways via which nuclear morphogenesis might proceed. First, we may consider a mechanism of *passive adaptation,* whereby the nucleus is forced to take a shape and a position that can be accommodated into a given cytoplasmic environment. Alternatively, nuclear morphogenesis may involve a mechanism of *autonomous differentiation,* whereby a program of morphogenetic changes unfolds concomitantly with, but independently from, the changes taking place in the cytoplasm. Finally, the nucleus may differentiate in coordination with the rest of the cell through a dynamic *feedback process.*

Apparently, the accumulation of organized product in the cytoplasm affects the positioning of the nucleus. This is evident in cells such as the mucous-secreting epithelia (goblet cells) and the adipocytes where the nucleus is "pushed" to one side of the cell close to the plasma membrane. However, this does not mean that the nucleus is always disposed at the periphery of the cell as product accumulates. For example, mast cells and leukocytes, whose cytoplasm is filled with granules, still possess a more or less centrally located nucleus.

It would be technically difficult to examine whether the nucleus has a potential to self-differentiate. The tight anchorage of this organelle to the cytoskeleton and its connections to the ER make it impossible to "uproot" a nucleus and transplant it undamaged to an enucleated host cell. However, the fact that transfection of somatic cells with a spermatocyte-specific lamin construct converts the nucleus from spherical to hook shaped suggests that the nucleus has the capacity to differentiate, to a certain degree, independently of the cytoplasm.

An approach to distinguish whether the nucleus is responsive to cytoplasmic cues would be to explore cell fusion experiments. Such heterokaryon experiments have been performed by several investigators. For example, fusion of intact chicken erythrocytes with whole HeLa or rat epithelial cells has yielded two interesting results: on one hand the "dormant" chicken nuclei have been reactivated when put in the cytoplasm of a relatively less differentiated cell; on the other hand, the mixing of the heterolo-

gous cytoplasms has been found not to affect the nuclear architecture of the host cell [reviewed in Lewin, 1980]. From such observations it can be inferred that even a terminally differentiated nucleus is responsive to cytoplasmic stimuli. However, by the same token one should also conclude that the nucleus does not passively adapt to a foreign cytoplasmic environment.

If there is indeed crosstalk between the cytoplasm and the nucleus, as these experiments suggest, how is this communication mediated, how does the nucleus probe the cytoplasmic environment around it? Based on recent findings, one can cite a number of interesting possibilities. First, a large number of soluble proteins seem to continuously shuttle between the nucleus and the cytoplasm [e.g., Borer et al., 1989]. Shuttling proteins may receive signals or be modified in a way characteristic of the state of the cytoplasm or the nucleus. This information could then be relayed back and forth as such "reporter" molecules shuttle between the two compartments. Second, several cytoplasmic proteins (including kinases) are targeted to the nucleus following activation by specific stimuli [e.g., Nigg et al., 1985]. Again, these molecules may "report" the state of the cytoplasm and the arrival of extracellular messages to the nucleus. Finally, it has now been documented that even intrinsic proteins of the inner nuclear membrane can shuttle in and out of the nucleus, probably by lateral diffusion throughout the entire system of endomembranes [Powell and Burke, 1990]. Apart from conveying information, shuttling membrane proteins may also participate in the remodeling of the nuclear surface. For instance, induction of a massive efflux of such proteins from the nucleus may limit the number of potential chromatin attachment sites on the nuclear envelope. Transient interactions with cytoplasmic components may also reorganize the cytoplasm around the nucleus.

FUTURE DIRECTIONS

Recognizing the potential for a continuous communication between the cell nucleus and the rest of the cell does not automatically mean that we have a good grasp of the mechanisms of nuclear morphogenesis. Elucidating a mechanism involves characterizing all the components responsible and understanding their dynamic inter-relationships. By such standards, we are still far from understanding the complex processes that lead to nuclear differentiation. However, this does not imply that any discussion on nuclear morphogenesis is necessarily premature. In fact, it is precisely the coming together of apparently unrelated data and "minor" or "forgotten" observations that allows us to formulate reasonable and testable ideas.

In this vein, it seems likely that the identification of the subunits of intranuclear filaments will undoubtfully advance the understanding of nuclear morphogenesis. Since some of the candidate proteins that may form nucleoskeletal fibers represent IF subunits (lamins) or coiled-coil proteins (NuMA), it would be interesting to screen DNA libraries with degenerate probes under low-stringency conditions and identify more IF-like proteins occurring in the nucleus. Another profitable approach may be the screening of directional expression libraries with IF probes. By anyone's measure, IF proteins represent an evergrowing superfamily and it is conceivable that new nuclear subunits await discovery. A traditional "nearest-neighbor" approach could be the method of choice to identify new lamin-binding proteins. This search may be facilitated by parallel studies aiming at the identification of substrates for nuclear envelope–bound kinases. Finally, the most important of all will be to pose new questions. For example, is the nucleus of a polarized epithelial cell also polarized? Are the IFs, the nuclear pores, and the nuclear envelope–associated structures asymmetrically or symmetrically arranged in the lobulated nuclei of white blood cells? Does chromatin binding affect the assembly of the nuclear lamina? Is the lamina locally modified during cytodifferentiation?

All of these questions (and many more) are central to a better understanding of nuclear architecture. To answer them we need vivid discussion and daring experiments. Maybe we should spend more time on the microscope and less on our PCs.

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