New and Notable

Regaining Eukaryotic Identity after Cell Division: Relax, Young Cell

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The appearance of the eukaryotic building plan during evolution, involving the intracellular segregation of genetic material and protein synthesizing apparatus, can be considered as a phase transition (1), opening a whole new world for multicellular developments including organisms with refined neuronal systems. Instrumental to the eukaryotic building plan is the nuclear envelope (for review, see Hetzer et al. (2)), which separates nucleus from cytoplasm. It confines most macromolecules, particles, and cell organelles to their cognate compartment, nuclear contents, or cytoplasm. At the same time, it is highly permeable for molecules needed in both compartments, such as inorganic ions, metabolites, and a host of small proteins. In addition, the nuclear envelope transfers the final products of transcription, such as mature messenger ribonucleoprotein particles and ribosomal subunits, with exquisite specificity and efficiency from nuclear contents to cytoplasm. Conversely, it specifically transports gene regulatory proteins from cytoplasm to nucleus. All these diverse transport functions are executed by a single transporter, the nuclear pore complex (NPC), a huge structure spanning the nuclear envelope and occurring in, usually, some thousand copies per cell. The eukaryotic building plan is challenged by cell division, because the nuclear envelope including NPCs is disassembled at the onset and reassembled at the completion of mitosis. Therefore, the question of how newborn cells reestablish the permeability barrier between genetic material and protein synthesizing apparatus, and thus rebuild eukaryotic identity, has captured the imagination.

Since the pioneering work of Swanson and McNeil (3), a rather simple picture has prevailed in this regard. Major functions such as transcription and protein synthesis are shut down at the onset of mitosis. The nuclear envelope is disassembled and chromosomes are condensed. Chromatides are separated and cytokinesis is initiated. In the daughter cells, chromosomes are tightly engulfed by double membranes derived from the endoplasmic reticulum. NPCs with full, interphaselike functionality are inserted into the new nuclear envelope. Basic functions are resumed, the nucleus is enlarged, and the cell cycle continues. However, two articles (4,5) suggest thought-provoking modifications of this scheme.

Both articles (4,5) report on the permeability of the nuclear envelope for photoswitchable proteins, i.e., proteins that can be converted from nonfluorescent to fluorescent states, and vice versa, by short light flashes. Shimozono et al. (4) used three such probes with Stokes radii of 1.8 (mKikGR), 4.0 (KikGR), and 5.2 nm (mECFP-KikGR), respectively, whereas Dultz et al. (5) used a probe (Dronpa) which is known to have a Stokes radius of 2.8 nm. Nucleocytoplasmic transport was measured by expressing the probes in mammalian cells, activating fluorescence in small cytoplasmic spots of individual cells by a laser flash, and monitoring the appearance of fluorescent probes in the nucleus by confocal scans. By deactivation of probe fluorescence after each flux measurement, in this issue, Dultz et al. (5) report they were able to measure transport repeatedly in the same cells.

The fluorescence photobleaching method was extended to membrane transport some time ago (6), and this type of compartmental analysis has recently become very popular under various labels (for review, see Rabut and Ellenberg (7)). The addition of photoswitchable proteins to the process now adds reversibility and repeatability to such flux measurements and thus lifts the technique to a new level of applicability.

The articles by Shimozono et al. and Dultz et al. (4,5) report univocally that the permeability of the newly formed nuclear envelope is relaxed, and that the more stringent exclusion limit of the interphase NPC is established only gradually within a time span of 2 h after cell separation. Thus, molecules with a Stokes radius of 4.0 nm, which cannot permeate through the mature NPC, easily enter newly formed nuclei. The relaxation of nuclear envelope permeability is size-dependent. The exclusion limit of the mature NPC is reached earlier for larger and later for smaller probes. The available evidence, though circumstantial, also suggests that the relaxation of nuclear envelope permeability is not due to gaps or holes in the nuclear envelope but reflects the properties of the newly synthesized nuclear envelopes. In contrast to the transport of inert molecules, the selective transport of proteins containing a nuclear localization signal seems to be fully effective at interphaselike strength from the very beginning. Thus, it is surprising that the requirements for rejecting cytoplasmic proteins from, and retaining nuclear proteins in, the nucleus seem to be less vital than thought.

The results (4,5) give rise to far-reaching questions. Does the NPC undergo a process of maturation after synthesis and insertion into the nuclear envelope? Is the maturation process different for the two transport modes of the NPC, hindered and facilitated diffusion? Are hindered and facilitated diffusion uncoupled from each other? Can hindered and facilitated diffusion be regulated independently of each other? Does the cell make use of the possibility to independently regulate

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hindered and facilitated diffusion to steer important cellular processes? Answers to these questions would shine new light on the vividly discussed transport mechanism of the NPC (8), the role of the NPC in cell regulation, and, most fundamentally, on the eukaryotic building plan.

Important questions have been raised but have yet to be answered. To answer them, it would be necessary to have measurements, or at least reasonable estimates, of the unitary transport coefficients of the NPC for both hindered and facilitated diffusion in relation to time after cell formation. In the simplest case, the transport coefficients depend not only on mean concentration changes in the involved compartments but also on compartment volumes and NPC numbers. In general, also, the time-dependent diffusion in nuclear volume and cytoplasm is involved. Among these parameters, the volumes of nuclear contents and cytoplasm and the numbers of NPCs change dramatically during a cell's youth, and so far, these changes have not been correlated with mean concentration changes. Thus, the studies by Shimozono and Dultz and their colleagues (4,5) are surprising and thought-provoking and indicate in which direction to proceed. However, as always, implicit in the new results is the need for more work.

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