The size-wise nucleus: nuclear volume control in eukaryotes

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Eukaryotic cells have an "awareness" of their volume and organellar volumes, and maintain a nuclear size that is proportional to the total cell size. New studies in budding and fission yeast have examined the relationship between cell and nuclear volumes. It was found that the size of the nucleus remains proportional to cell size in a wide range of genetic backgrounds and growth conditions that alter cell volume and DNA content. Moreover, in multinucleated fission yeast cells, Neumann and Nurse (see p. 593 of this issue) found that the sizes of individual nuclei are controlled by the *relative* amount of cytoplasm surrounding each nucleus. These results highlight a role of the cytoplasm in nuclear size control.

One of the fundamental properties of eukaryotes is their ability to maintain cell sizes and organelle volumes that are appropriate for different growth and differentiation states. Although most organelles, such as the ER and mitochondria, can vary greatly in amounts, it has long been observed that cells maintain a roughly constant "karyoplasmic ratio" (the ratio of the nuclear volume to cell volume) (Wilson, 1925; Cavalier-Smith, 2005). This volume relationship is found in cells with widely different DNA contents, ranging from single-celled eukaryotes to mammalian cells. Now, a more penetrating look at this question has been taken in two studies involving quantitative morphometric analysis of yeast (Jorgensen et al., 2007; Neumann and Nurse, 2007).

The nucleus is known to increase in volume through the cell cycle (for review see Umen, 2005), but how this is coupled to cell cycle progression and cell growth is largely mysterious. The cell size checkpoint in budding yeast requires that cells reach a critical size for S phase progression (Umen, 2005). By examining mutants of budding yeast that enter into S phase at a cell size smaller than normal, Jorgensen et al. (2007) observe that the nuclear/cell (N/C) volume nonetheless remains constant in asynchronously growing populations, with the nuclear volume occupying ~8% of the cellular volume. Their studies suggest that nuclear volume increases throughout the cell cycle in concert with cell growth, and does not take place discontinuously at points such as the onset of S phase.

Related findings were made by Neumann and Nurse in fission yeast (see p. 593), using mutants that enter mitosis at a smaller than normal cell size. The nuclei turn out to be correspondingly smaller at the time of mitosis, thereby maintaining a normal N/C volume ratio of ~0.08. Mutant strains where mitosis was uncoupled from S phase, which gave rise to cells with up to 16-fold higher DNA content, still maintained a similar N/C ratio.

Neumann and Nurse (2007) then performed a real-time imaging of cells labeled with a GFP-tagged nuclear envelope (NE) marker to directly track nuclear and cellular volume increases during the cell cycle. First, they demonstrated that cell nuclei undergo a *continuous* increase in volume from G1 until M phase, extending the conclusions of Jorgensen et al. (2007). They also examined mutant strains blocked in cytokinesis, in which cells acquired multiple, unevenly distributed nuclei. In this situation, nuclei with a range of volumes were present in a single cell. Interestingly, the volume of each nucleus was directly proportional to the amount of "surrounding" cytoplasm. The nuclei located in central regions of these cells were crowded into a relatively small cytoplasmic area and were proportionally smaller than the peripheral nuclei, which occupied a greater cytoplasmic space.

To investigate the mechanisms of size control in these multinucleated cells, the authors mechanically displaced nuclei in the cells by centrifugation, and then evaluated the growth of individual nuclei in real time. Nuclei that were positioned within a disproportionately large amount of surrounding cytoplasm grew more rapidly than in normal mononucleated cells, up to the point where a N/C ratio of ~0.08 was achieved. By contrast, nuclei surrounded by small amounts of cytoplasm "waited" until the adjacent cytoplasmic volume became sufficiently large before they started growing. These results strongly suggest that cytoplasmic components directly and "dominantly" influence nuclear growth in fission yeast. An effect of the cytoplasm on nuclear size also was seen with metazoan systems, showing that the highly condensed avian erythrocyte nucleus undergoes dramatic swelling when fused to a proliferating cell (Harris, 1967), and that the heterochromatic nucleus of sperm grows continuously when introduced into the cytoplasm of *Xenopus* oocytes in vitro (Gurdon, 1976). The experiments with multinucleated yeast cells indicate that nuclear size control does not involve a diffusible cytoplasmic factor; otherwise, all nuclei within the

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Abbreviations used in this paper: N/C, nuclear/cell; NE, nuclear envelope.

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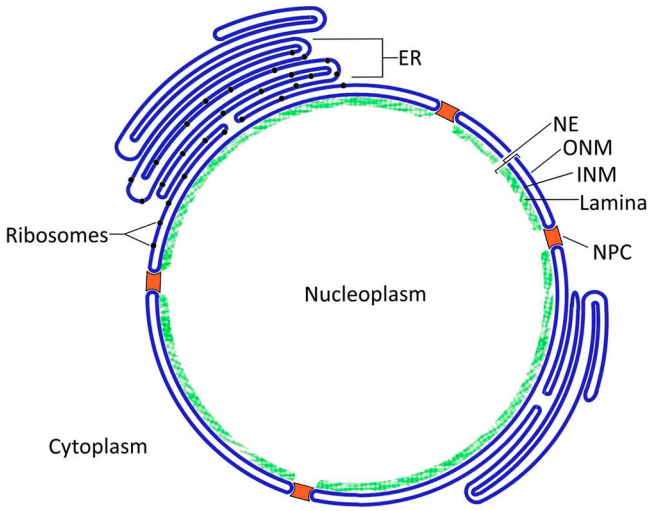

Figure 1. **Schematic representation of the nuclear envelope/endoplasmic reticulum system of metazoan cells.** The nucleoplasm is enclosed by the nuclear envelope (NE), which contains the inner nuclear membrane (INM), the outer nuclear membrane (ONM), the nuclear pore complex (NPC), and the lamina. The INM and ONM are continuous at the nuclear pores, and the ONM is continuous with the endoplasmic reticulum (ER), potentially allowing the flux of lipids and proteins to the inner and outer nuclear membranes during nuclear expansion. The yeast NE also contains NPCs and is continuous with the peripheral ER, but a functional counterpart to the metazoan lamina has not yet been described in yeast.

cell would have the same volume. This suggests that nondiffusible structural components of the cell are important for dictating nuclear size. A priori, such a size integrator could involve the cytoplasmic cytoskeleton because the NE is physically connected to cytoplasmic actin filaments and microtubules in metazoans (Starr and Fischer, 2005). However, dynamic actin filaments and microtubules do not appear to influence nuclear size in fission yeast because drugs that affect these structures have no effect on N/C ratios (Neumann and Nurse, 2007).

An alternative possibility is that nuclear size control involves the membrane skeleton of the peripheral ER and/or NE. At a fundamental level, nuclear volume is determined by the size and shape of the NE (Fig. 1). In higher eukaryotes, it is clear that nuclear size (Newport et al., 1990; Yang et al., 1997) as well as shape (for review see Gruenbaum et al., 2005; Worman and Courvalin, 2005) is strongly influenced by the nuclear lamina, a protein meshwork lining the inner nuclear membrane that consists of a polymer of nuclear lamins associated with other, more minor proteins. Nonetheless, the molecular basis for the involvement of lamins in nuclear size and shape is unclear. Because homologues of lamins are not present in yeast, nuclear size control in these organisms must involve other proteins.

Whereas metazoan cells have an open mitosis that involves disassembly and subsequent reformation of the NE and lamina, yeast undergo a closed mitosis in which the NE remains intact. In both higher and lower eukaryotes, the peripheral ER is morphologically continuous with the outer nuclear membrane (Fig. 1). During yeast mitosis, the peripheral ER–NE connection could be central for the increase in NE surface area that occurs when two small daughter nuclei are formed from a parent nucleus (Neumann and Nurse, 2007). In this case, the peripheral ER could provide a membrane reservoir that becomes readily incorporated into the NE by lipid and protein flow (Fig. 1). This invites the question of why peripheral ER membranes remain structurally segregated from the NE during interphase. Is it possible that "barrier" structures, such as the nuclear pore complex, restrict the movement of lipid to the NE at certain periods of the cell cycle? Do yeast have a functional counterpart of the metazoan nuclear lamina to help specify the structure of the NE? Do they contain a membrane skeleton in the peripheral ER that prevents it from merging with the NE during interphase?

Ultimately, these considerations raise the question of why nuclear size and shape are so carefully controlled in eukaryotes. Nuclear size and shape become aberrant in cellular states like cancer (Zink et al., 2004) and with certain protein mutations (Santos-Rosa et al., 2005; Worman and Courvalin, 2005; Brandt et al., 2006). When does aberrant nuclear structure contribute to cellular dysfunction, and when is it simply a consequence of it? Although many different factors are likely to be involved in nuclear size control in eukaryotes, the tools of genetics and cell biology should soon begin to expand on this issue.

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