Mitosis is swell

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Cell volume and dry mass are typically correlated. However, in this issue, Zlotek-Zlotkiewicz et al. (2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201505056) and Son et al. (2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201505058) use new live-cell techniques to show that entry to mitosis coincides with rapid cell swelling, which is reversed before division.

How growth is linked to division by the cell cycle regulatory network is an important open question in cell biology (Turner et al., 2012; Ginzberg et al., 2015). Yet, what is meant by cell growth? Different methods have been used to estimate either the total dry mass of the cell, total protein content, or cell volume. Although these parameters are often highly correlated, they are not the same. In budding yeast, growth parameters are nearly interchangeable as cell density changes only about 1% through the division cycle (Bryan et al., 2010). In contrast, cell density can drop by over 50% during a rapid growth phase in hypertrophic chondrocytes, which are responsible for determining bone length (Cooper et al., 2013). However, because of the current lack of similarly dramatic examples, it is assumed that chondrocytes are a special case and that most animal cells also exhibit little variation in cell density, as recently measured (Bryan et al., 2014). Yet this assumption has not been thoroughly tested because of the difficulty of measuring cell volume in animal cells, which are often irregularly shaped.

Measuring cell volume is even more challenging in live cells. Whereas there is an accurate live-cell method for measuring dry mass in quantitative phase microscopy (Sung et al., 2013), live-cell volume measurements of adherent cells have been difficult because of their irregular geometry. Current methods are mostly based on 3D geometric reconstructions from confocal sections. However, confocal microscopy has poor resolution in the z-dimension, and increasing the number of z-sections to better estimate the cell membrane location and improve accuracy can be phototoxic.

In this issue, Son et al. and Zlotek-Zlotkiewicz et al. applied two different methods to accurately measure cell volume changes in live cells. Son et al. (2015) used a variation of the suspended microchannel resonator pioneered by the Manalis laboratory (Fig. 1 A; Burg et al., 2007). In this method, the resonance frequency of the device shifts when a cell enters a part of a microchannel because the cell is of a different density than the surrounding media. The change in resonance frequency can therefore be used to calculate the buoyant mass of the cell. Changing the media of the microchannel to one of different density and then performing

the same measurement for the same cell allows the accurate calculation of both cell dry mass and volume. One limitation of the microchannel resonator method is that the cells are required to be nonadherent so that they can be moved into and out of the resonator. To measure cell volume of adherent cells, Zlotek-Zlotkiewicz et al. (2015) used a microchamber culture device with a low 15–25-µm adjustable ceiling (Fig. 1 B). Cells were grown in a media containing fluorescent dye–labeled dextran. Cell volume could then be measured from epifluorescence images because the cells displaced the fluorescent dextran in proportion to their volume. This method was combined with quantitative phase microscopy to measure dry mass.

Both Son et al. (2015) and Zlotek-Zlotkiewicz et al. (2015) applied their methods to precisely and noninvasively measure the volume and density dynamics in growing and dividing mammalian cells (Fig. 1 C). During most of the cell cycle, density is constant and dry mass is correlated with volume. However, the researchers found that cell volume, but not dry mass, increases rapidly as cells enter mitosis. This osmotic swelling occurs during prophase and prometaphase before being reversed in anaphase and telophase. Collectively, the work of both teams also determined that mitotic swelling is driven by osmotic water exchange and requires the activity of the Na/H ion exchanger but is not dependent on the actomyosin cortex, endocytosis, or cytokinesis. Whereas previous studies gave contradictory results, the two papers in this issue show that there is a reversible 10–30% volume increase during mitosis depending on the type of cell.

The establishment of cell swelling during mitosis raises the question of its function. In laboratory conditions, mitotic animal cells lose surface adhesion and are spherical. This spherical geometry is accompanied by an increase in intracellular hydrostatic pressure (Stewart et al., 2011). In the in vivo context of an animal tissue, an increase in intracellular pressure accompanied by cell swelling would allow cells to push against their neighbors and open up additional space for mitosis (Fig. 1 D; Son et al., 2015; Zlotek-Zlotkiewicz et al., 2015). The mitotic acquisition of a larger, more spherical geometry may be important because physically preventing cells from rounding up retards mitosis and promotes inaccurate chromosome segregation (Lancaster et al., 2013). Alternatively, the dilution of the cytoplasm by swelling might change the physicochemical properties of the intracellular environment to facilitate chromosomal movement and segregation or change the kinetics of biochemical reactions (Son et al., 2015).

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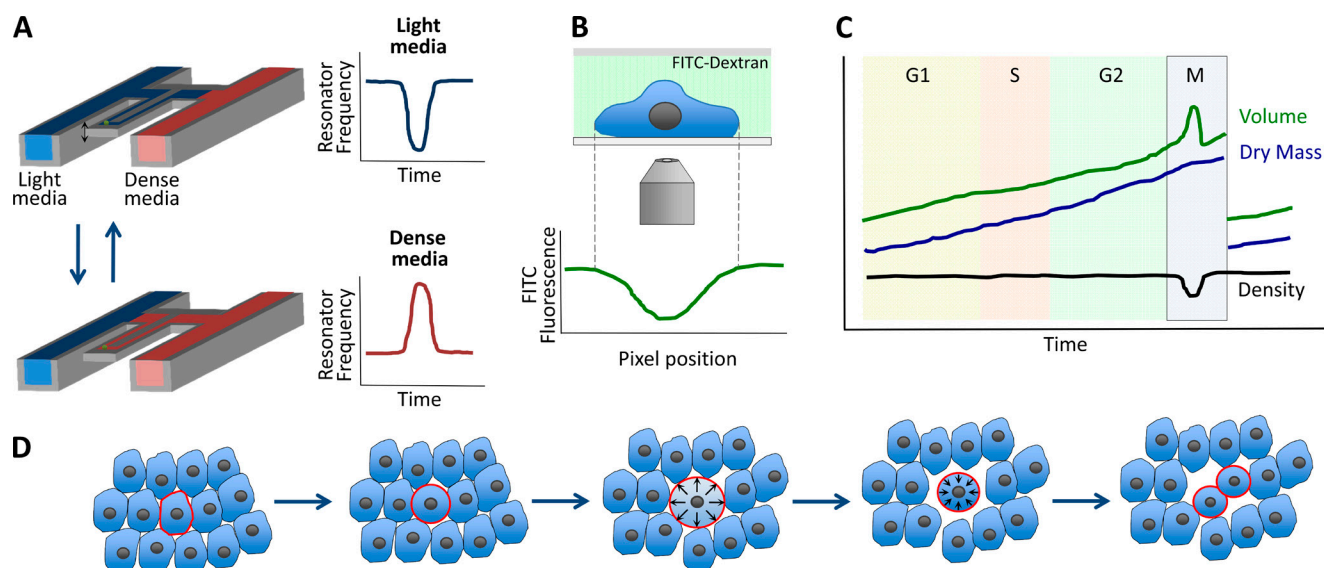
Figure 1. Two new live-cell measurements of cell volume and mass reveal that cells swell in mitosis. (A) Schematic of microchannel resonator whose frequency is determined by the cells' buoyant mass. Live-cell measurements in two media of different density allow calculation of cell volume and density (modified from Son et al., 2015). (B) Using epifluorescence microscopy, cell volume can be measured as the amount of dye-labeled dextran displaced in a low-ceiling culture chamber. (C) Cell density is constant through the cell cycle except in mitosis, when cells swell (modified from Son et al., 2015). (D) In the context of an animal tissue, mitotic swelling may generate a larger, rounder space to promote accurate and rapid chromosome segregation.

Live-cell methods that accurately measure volume will most obviously be useful for studies of how cell growth is linked to cell cycle progression but are unlikely to be limited to this application. For example, it would be interesting to follow the dynamics of cell volume and density in other processes in which the surface area to volume ratio can change rapidly, such as cell migration (Traynor and Kay, 2007). Depending on the environment, cells can switch from actin-driven motility to hydrostatic pressure–driven bleb-based motility (Sahai and Marshall, 2003; Zatulovskiy et al., 2014). Because this motility switch strongly depends on the osmolarity of the environment (Fedier and Keller, 1997; Yoshida and Soldati, 2006), it is likely to be accompanied by and perhaps even require cell swelling.

Although the swelling of animal cells has been mostly neglected, cell swelling is not unusual in other eukaryotic lineages. Unlike animal cells, which have a flexible cell geometry that can rapidly be remodeled, plant and fungal cells have a stiff cell wall and cannot easily change their geometry. Nevertheless, plants and fungi can use regulated swelling to move on time scales faster than that of growth (Skotheim and Mahadevan, 2005). For example, a stem bending to track the sun is caused by cells on one side swelling, whereas those on the other side shrink. This differential swelling allows the stem to bend because the plant tissue is connected by elastic cell walls. Using such differential swelling, plants and fungi can perform impressive coordinated movements to track the sun, compete for territory, disperse seeds or spores, and catch prey (Attenborough, 1995). Although swelling-based movements have long been appreciated in the context of plants, there is no a priori reason animal cells might not also harness such mechanisms to perform important functions. The further development and dissemination of technologies to accurately measure cell volume, density, and dry mass, such as those described in this issue, will be essential to determine the extent to which animal cells harness swelling.

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