Does size matter?

For 40 years, the debate has raged. Do mammalian cells monitor cell size when deciding whether to divide? More recent models suggest an indirect solution, but the field is far from reaching a final verdict.

The cell cycle has provided us with some of the most spectacular examples of evolutionary conservation, with several key enzymes and entire regulatory cassettes being interchangeable between yeast and mammals. But when it comes to the control of growth and division in G1, the story is a little different.

G1 is the critical phase during which both budding yeast and mammalian cells commit to another cycle. The question is whether both cell types take growth, as measured by cell size, into account when making the decision to divide.

"The classical view in yeast, more or less completed by 1980, is that growth is just growth, making more mass, and then there is a critical checkpoint where that is monitored, probably by G1 cyclin synthesis," says Fred Cross (Rockefeller University, New York, NY). "Those experiments are pretty good. In mammalian cells it is less clear that this view applies."

The yeast work showed that whereas growth continues during cell-cycle delays, the cell cycle halts during growth delays (Johnston et al., 1977). That put the emphasis on cell size checkpoints. But now researchers such as Ian Conlon and Martin Raff (University College, London, UK) are arguing that mammalian cells rely only on a balance of progrowth and prodivision extracellular signals to make their G1 decisions.

A different story came from Bruce Futcher (State University of New York, Stony Brook, NY) at the recent FASEB conference on Receptors and Signal Transduction (Salt Lake City, Utah, June 29–July 3). He claimed that yeast and mammalian cells might resemble each other more than has been thought. "In the bits where we understand quite well what's happening, they are very similar," he says. "The bits where people imagine they are different are all bits where we don't really understand what is happening."

From yeast to mammals

Futcher has promoted a model in which the yeast G1 cyclin Cln3p accumulates at a rate that is determined by the volume of cytosol. As the cytosol increases in size, more Cln3p accumulates but the size of the nucleus stays relatively constant, leading to a higher concentration of Cln3p in the nucleus. This eventually propels the cell through the cell cycle decision point, which in yeast is called START (Hartwell and Unger, 1977).

In his talk, Futcher showed that increasing the size of the nucleus by 10% (by adding a prolific but innocuous plasmid) led to an 8% increase in critical size. The smallest cells to bud in this experiment were almost all plasmid-negative.

Do mammalian cells monitor their size?

Similarly detailed models have not been forthcoming in mammalian cells. But some early experiments did indicate that size is important. Anders Zetterberg (Karolinska Institute, Stockholm, Sweden) found in 1965 that the postmitotic mass of 21 populations of mouse fibroblasts correlated with the length of their G1 period, with smaller cells having a longer G1 (Killander and Zetterberg, 1965) (Fig. 1). As a result, small and large cells entered S phase at almost the same cell size, which made these mammalian cells look a lot like yeast. On the basis of these and later experiments, Zetterberg says that "there is probably some kind of active size control."

When, in mammalian cells, would such a size control operate? Zetterberg's later experiments indicated that the mammalian restriction point—the transition from mitogen-dependent to mitogen-independent growth—is not equivalent to yeast START, since it is not the point at which growth is assessed. Zetterberg found that the period up to the restriction point was invariant, with the size-dependent variation in timing coming during G1 but after the restriction point.

Further evidence suggestive of size control was reported at the Utah conference by Wei-Xing Zong (University of Pennsylvania, Philadelphia, PA), who spoke about work performed by Marian Harris in Craig Thompson's laboratory. Harris used cells that, because they lack two key apoptotic proteins, no longer die when starved of interleukin 3 (IL-3) for a month. When IL-3 was added back to these suspended animation cells, they started increasing in size almost immediately. But they did not enter S phase for 10–14 d, by which time their cell size was almost equal to that of normal cycling G1 cells. Again, this looks like size is monitored during the cell cycle. The cells may, however, be rebuilding certain essential organelles or cellular structures during the lag period.

When it comes to the more direct, Zetterberg-like experiments, others have found either the presence (Yen et al., 1975) or absence (Fox and Pardee, 1970) of correlation between size and G1 duration. "It's still never been settled whether there is a critical size for mammalian cells," says Jim Roberts (Fred Hutchinson Cancer Research Center, Seattle, WA). "In different kinds of cells you get different answers." Most cell types present one or another problem for these experiments. Some transformed cells may lack certain cell cycle controls, and primary cells tend to be adherent and thus difficult to size accurately. For now, Roberts doubts that the phenomenon exists: "I would say, go back to the original work and prove to me that there is size control."

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Dispensability and randomness

This view is seconded by Robert Brooks (King's College, London, UK). Initially, he was concerned that, without a cell size checkpoint, any size difference in a population might be amplified by continued, runaway growth. But, he says, "that sort of thing is true only if growth is exponential. If that isn't the case, you don't absolutely have to have a size control."

Exponential growth (not to be confused with exponential division) means an increase in the rate of mass accumulation during the cell cycle. "In yeast, growth looks more compellingly exponential," says Brooks. "In mammalian cells, this is not the case." Indeed, Brooks found that 3T3 cells that start off larger actually grew more slowly than their smaller counterparts, suggesting that cell size would return to a mean without the need for a cell size checkpoint (Brooks and Shields, 1985). This conflicts, however, with Zetterberg's demonstration that the rate of RNA and mass accumulation in mouse fibroblasts increases as interphase progresses (Zetterberg and Killander, 1965).

Return to a mean is compatible with a G1 transition governed not by size but by a random probability event. Brooks and colleagues put forward this idea to explain the variability in timing of S phase onset in 3T3 cells (Shields et al., 1978), and Brooks sees a version of it in his current experiments, in which different nuclei in a single frog extract enter S phase at different times (Hola et al., 1996). The nature of this nuclear-localized trigger remains elusive.

The extracellular camp

Raff sees the concept of a cell size checkpoint in mammalian cells as both unnecessary, based on the lack of exponential growth, and unlikely, based on the differing biology between yeast and mammalian cells. "I'm pretty confident that you don't need it and that the cells that we have looked at don't have it," he says.

Conlon and Raff have focused on the effect of extracellular signals. They have found that IGF-1 promotes cell growth and cell cycle progression of rat Schwann cells, but glial growth factor (GGF) promotes only cell cycle progression. Increasing GGF leads to more rapid division and progressively smaller cells, suggesting that the cells are not held up by an intracellular G1 program that measures size (Conlon et al., 2001).

"The field has bought the idea of size control, hook, line, and sinker, on the basis of yeast experiments," says Raff. But yeast cells are limited by the level of nutrients in the medium, whereas the cells of multicellular animals, swimming in a soup of nutrients, have their growth limited mainly by extracellular signals. Regulation based on these signals would allow cell size to be controlled on an organismal rather than single cell level. "This is very different from yeast cells," says Raff. "A fundamental difference."

Conlon and Raff point to their recent experiments in which Schwann cells were shifted between different growth conditions. The cells took over a week to reach their new characteristic size, suggesting a gradual, growth-driven process rather than a strict cell size checkpoint. By contrast, fission yeast cells subject to similar shift experiments (Fantes and Nurse, 1977) undergo rapid, single cycle changes that support the notion of a cell size checkpoint.

Measure rate not size

There may, however, be a middle ground. For starters, some researchers believe that both types of cells may be responding to levels of intracellular nutrients. In yeast, those intracellular levels may directly reflect the levels of extracellular nutrients, whereas in mammalian cells the intracellular levels may be determined by transporters regulated by Raff's extracellular signals. "As always in evolution," says Mike Tyers (University of Toronto, Canada), "it is a question of building on additional regulatory layers."

Then, there is the translation argument. Several investigators propose that, rather than directly gauging cell size, cell cycle regulators are especially sensitive to translation capacity and that translation capacity is thus used as a way to communicate growth levels to the cell cycle.

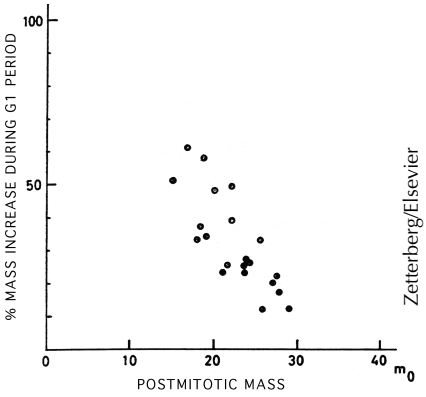

Figure 1. **Cells that start off G1 larger gain less mass during G1.** Correlations such as this led Zetterberg to propose that size control applies in mammalian cells (see text).

The clearest example of this again comes from yeast work. Emmett Schmidt (Massachusetts General Hospital, Charlestown, MA) has found that the budding yeast gene encoding Cln3p has an upstream open reading frame (uORF) that represses *CLN3* expression. The uORF makes successful *CLN3* expression (as measured by polysomal profiles and not, unfortunately, by Cln3p levels) dependent on a high concentration of ribosomes (Polymenis and Schmidt, 1997). A similar mechanism may operate in fission yeast, although in this case the cell cycle component with exceptional translation sensitivity is Cdc25, a phosphatase that controls entry into mitosis (Daga and Jiminez, 1999).

This coupling between bulk synthesis rate and cell cycle components is similar to that proposed by Bruce Edgar (Fred Hutchinson Cancer Research Center, Seattle, WA) for a beast that is, if not mammalian, then at least multicellular. For flies, says Edgar, "there is a measurement mechanism, but I don't think it's measuring size. My opinion, and it's really an opinion, is that protein synthesis rates can affect cell division rates, and in particular rates of G1/S progress. A cell with a higher protein synthesis rate

would have a higher rate of initiating G1/S transitions. That would indirectly give you a cell size measuring mechanism."

To support such a model, Edgar has introduced growth promoters such as Myc, Ras, and components of the insulin pathway. Myc, in particular, increases both ribosome biogenesis and translation rates. Levels of a critical G1 cyclin called cyclin E are also increased. This may be mediated by an increase in protein stability, but an alternative explanation is that the increased translation rates combat the constitutive instability of the cyclin E protein. Thus, this increase in translation could provide the critical boost that links growth to cell cycle progression.

These models rely on cell cycle transcripts being second class citizens that are not translated until a critical level of ribosomes is available. Consistent with this idea, several experiments suggest that mammalian cells take care of growth before tackling division. S6 kinase, for example, directs preferential translation by sending ribosomes to the oligopyrimidine tracts at the 5′ end of ribosomal protein mRNAs. This system first churns out more raw materials for ribosomes, and only when those excess ribosomes have been created will the cell have the capacity to move onto other mRNAs, such as those encoding cell cycle regulators. George Thomas (Friedrich Miescher Institute, Basel, Switzerland) uses this argument to explain why his S6 kinase mutant flies have smaller cells (Montagne et al., 1999), since they have lost this control and thus make excessive cell cycle regulators that drive division over growth.

Thomas has further evidence for growth before division in mammalian cells. In mouse liver cells, he has prevented increases in ribosome numbers with a conditional knockout of the S6 ribosomal protein and then subjected those cells to tests of either growth or division. The cells were able to use their limited number of ribosomes to grow in size after starvation and refeeding but were unable to divide to replace liver mass that had been removed by surgery (Volarevic et al., 2000). Thus, once again, growth can be turned on more easily than cell division.

Then, there is the TOR connection. The target of rapamycin (TOR) is the focal point for a web of pathways that regulate growth in response to nutrient availability. But rapamycin, a TOR inhibitor that shuts down only a small subset of translation, halts not only growth but also entry into S phase. This suggests that TOR uses its assessment of nutrients and growth to regulate both growth and division. "I think rapamycin is the best evidence that there is a link here," says Nahum Sonenberg (McGill University, Montreal, Canada).

Growth anticipation

One of the peculiarities of the growth-and-division field is the distribution of the literature. After some careful experiments starting in the 1960s, there has been a long silence. A major reason for the hiatus was that attention was focused primarily on the cell cycle, but in yeast there was another factor.

"There has been a two decade interruption, really for the want of cloning whi mutants," says Tyers. It is easy enough to isolate yeast mutants that are small (wee in fission yeast, for their isolation in Edinburgh; whi in budding yeast, for a bet over a bottle of Irish whiskey), but there is no selection to clone the relevant genes. Recently Tyers got around this problem by testing the cell sizes of the complete set of ~6,000 budding yeast deletion strains (Jorgensen et al., 2002). This unbiased search gave him a clear answer. "Basically yeast are bags of ribosomes that must coordinate with the cell cycle," he says. "Our view of START has changed a lot over the past year."

The ribosome-centric view arose because the clearest size mutants had defects in ribosome synthesis. But they were not suffering from a general incapacitation—rather, they were uncoupled for growth and division, with their growth capability far more compromised than their division capability.

So far, this story sounds consistent with the S6 experiments, and Tyers agrees that "it's a very simple idea to view the cell cycle commitment step as a passive output of translation." But Tyers believes in a more active and direct mechanism in which ribosome biogenesis is monitored. Some of his deletions are in genes that function very early on in ribosome biogenesis. "The fact that these factors are clearly not functioning as part of the ribosome but as part of an assembly pathway leads to the argument that it is an anticipatory change," he says.

So what does this pathway tell the cell cycle? The Whi phenotype of Tyers' mutants gives a surprising answer—it suggests that the normal versions of these factors promote both ribosome biogenesis (more growth) and cell cycle inhibition (less division, as mutation causes more division leading to the smaller size).

This reverse coupling of growth and division seems counterintuitive for homeostatic growth, but it might be perfect for adjusting to different environments. It fits, for example, with the simple observation that budding yeast cells are larger on rich media. And Futcher has found that G1 cyclins are less abundant when cells are grown on poor carbon sources, which is when they go through START at a smaller size. This phenotype (less cyclins, lower critical size) could be seen as contradicting the models in which cyclins measure size and act as the threshold for division, but Futcher prefers to see it as an adjustment mechanism. "It would be an awfully poor and stupid cell," he says, "if it couldn't adjust for the things in its environment."

Once the threshold is adjusted and nutrient supply is stable, the relative concentration of a cyclin may still coordinate cell size with the G1 to S transition. But the resetting during changing growth conditions may rely on Tyers' progrowth, antidivision pathway.

Pathway profusion

Futcher's original point was that both yeast-like and mammalian-like pathways may exist in both cell types. Mammalian cells, like yeast, may monitor growth to influence cell division. And yeast cells, like mammalian cells, may use extracellular factors (such as glucose) to send signals directly to the cell-cycle machinery. Evidence for

the latter pathway comes, for example, from the glucose-dependent *CLN3* transcription factor Azf1p (Newcomb et al., 2002).

The relative importance of these pathways may differ in different organisms and cell types, and clearly it will take many more years to decipher which components are defining the critical set point for a given cell type. Cross feels that older models—in which growth and division were cleanly separated—may be supplanted by messier but more realistic wiring diagrams. "In the end," he says with a laugh, "there will no doubt be arrows pointing all over the place in some incomprehensible manner."

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References

Brooks, R.F., and R. Shields. 1985. Cell growth, cell division and cell size homeostasis in Swiss 3T3 cells. *Exp. Cell Res.* 156:1–6.

Conlon, I.J., G.D. Dunn, A.W. Mudge, and M.C. Raff. 2001. Extracellular control of cell size. *Nat. Cell Biol.* 3:918–921.

Daga, R.R., and J. Jiminez. 1999. Translational control of the Cdc25 cell cycle phosphatase: a molecular mechanism coupling mitosis to cell growth. *J. Cell Sci.* 112:3137–3146.

Fantes, P., and P. Nurse. 1977. Control of cell size at division in fission yeast by a growth-modulated size control over nuclear division. *Exp. Cell Res.* 107: 377–386.

Fox, T.O., and A.B. Pardee. 1970. Animal cells: noncorrelation of length of G1 phase with size after mitosis. *Science.* 167:80–82.

Hartwell, L.H., and M.W. Unger. 1977. Unequal division in *Saccharomyces cerevisiae* and its implications for the control of cell division. *J. Cell Biol.* 75: 422–435.

Hola, M., M. Howard, F.N. Nawaz, S. Castleden, and R.F. Brooks. 1996. Individual nuclei differ in their sensitivity to the cytoplasmic inducers of DNA synthesis: implications for the origin of cell cycle variability. *Exp. Cell Res.* 229:350–359.

Johnston, G.C., J.R. Pringle, and L.H. Hartwell. 1977. Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae. Exp. Cell Res.* 105:79–98.

Jorgensen, P., J.L. Nishikawa, B.-J. Breitkreutz, and M. Tyers. 2002. Systematic identification of pathways that couple cell growth and division in yeast. *Science.* 297:395–400.

Killander, D., and A. Zetterberg. 1965. A quantitative cytochemical investigation of the relationship between cell mass and initiation of DNA synthesis in mouse fibroblasts in vitro. *Exp. Cell Res.* 40:12–20.

Montagne, J., M.J. Stewart, H. Stocker, E. Hafen, S.C. Kozma, and G. Thomas. 1999. *Drosophila* S6 kinase: a regulator of cell size. *Science.* 285:2126–2129.

Newcomb, L.L., D.D. Hall, and W. Heideman. 2002. AZF1 is a glucose-dependent positive regulator of *CLN3* transcription in *Saccharomyces cerevisiae. Mol. Cell. Biol.* 22:1607–1614.

Polymenis, M., and E.V. Schmidt. 1997. Coupling of cell division to cell growth by translational control of the G1 cyclin *CLN3* in yeast. *Genes Dev.* 11: 2522–2531.

Shields, R., R.F. Brooks, P.N. Riddle, D.F. Capellaro, and D. Delia. 1978. Cell size, cell cycle and transition probability in mouse fibroblasts. *Cell.* 15:469–474.

Volarevic, S., M.J. Stewart, B. Ledermann, F. Zilberman, L. Terracciano, E. Montini, M. Grompe, S.C. Kozma, and G. Thomas. 2000. Proliferation, but not growth, blocked by conditional deletion of 40*S* ribosomal protein S6. *Science.* 288:2045–2047.

Yen, A., J. Fried, T. Kitahara, A. Strife, and B.D. Clarkson. 1975. The kinetic significance of cell size. *Exp. Cell Res.* 95:295–302.

Zetterberg, A., and D. Killander. 1965. Quantitative cytochemical studies on interphase growth. *Exp. Cell Res.* 39:22–32.