[Cell Cycle 6:2, 218-224, 15 January 2007]; ©2007 Landes Bioscience

Report

Cell Size Regulation in Mammalian Cells

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Original manuscript submitted: 12/12/06
Manuscript accepted: 12/24/06

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=1324

KEY WORDS

cell growth, cell cycle, growth factors, mitogens, cell size

ACKNOWLEDGEMENTS

We thank D. Parkinson, A. Woodhoo, M. Raff, S. Leevers, B. Baum and the Lloyd lab for their help. This work was supported by a FEBS grant to P.E. A.C.L. is a CRUK Senior Cancer Research Fellow.

ABSTRACT

The regulation of cell growth and proliferation is fundamental for animal development and homeostasis but the mechanisms that coordinate cell growth with cell cycle progression are poorly understood. One possibility is that "cell-size checkpoints" act to delay division until cells have achieved a minimal size or mass however, the existence of such checkpoints in mammalian cells is controversial. In this study we provide further evidence against the operation of a size checkpoint in mammalian cells. We show that primary mammalian cells proliferate at a rate that is independent of cell size or cell mass and that cell size is "set" by the balance of extracellular growth factors and mitogens. Moreover, we show that commonly used culture conditions stimulate cell growth much more than cell cycle progression resulting in cells that proliferate at sizes 300–500% larger than their in vivo counterparts. This has profound effects on cell behaviour.

INTRODUCTION

Cell growth and cell division appear to be precisely coordinated in proliferating cells. Thus, proliferating cells usually double their mass before each division, maintaining a constant mean size over time. The mechanisms coordinating these processes however, remain poorly understood. In yeast, there is evidence suggesting that growth regulates cell division by means of an intrinsic mechanism that monitors the size of the cell. This internal sensor, termed a size checkpoint, is thought to regulate cell size by pausing cell cycle progression until cells reach a minimum or critical size. However, as yeast cells divide at different sizes, depending on nutrient conditions, it means that, if this "critical size" exists, it must be adaptable.[1-4]

Recent studies have argued for and against the existence of size-checkpoints in animal cells and it remains controversial as to whether growth regulates cell cycle progression in multicellular organisms.[4-7] In yeast, growth rate increases with the size of the cell, so that bigger cells grow faster than small cells, a situation that would seem to require a cell size checkpoint to maintain a constant size distribution of the population over time.[8] Counter-intuitively, in mammalian cells, growth rate seems not to increase with cell size.[6] Theoretically, this linear control of cellular growth makes an internal size-checkpoint mechanism unnecessary.[6,8] There are other differences between growth control in yeast and mammalian cells: in animals, cell growth and cell proliferation are not solely determined by nutrient availability, as in yeast, but also by extracellular growth factors and mitogens.[3] This dependence on extracellular factors has been shown to be absolute: cells cultured in the absence of mitogens will not proliferate, and cells cultured in the absence of growth factors will not grow and despite being bathed in nutrients, will undergo autophagy and ultimately die.[9] Furthermore, growth and proliferation can clearly be regulated independently in animal cells. This can be seen both in vitro, where distinct factors have been shown to be able to regulate these processes separately,[10,11] and in vivo, where growth can occur in the absence of proliferation (nerve and muscle cells) and proliferation can occur in the absence of growth (blastomeres).[12] None of this evidence is conclusive however, and it remains uncertain how mammalian cell size is regulated.

MATERIALS AND METHODS

Cell culture. Schwann cells and perineural fibroblasts were purified from P7 rat sciatic nerve by immunopanning.[13] Schwann cells were expanded on dishes coated with PLL and laminin in serum-free (SF) medium (DMEM containing 100 μg/ml transferrin, 100 μg/ml

BSA, 16.1 μg/ml putrescine, 39 ng/ml selenium, 1 μM forskolin, 10 μg/ml insulin and 20 ng/ml NRG (R&D)). For the shift experiments Schwann cells were transferred to a serum-containing (SC) medium (DMEM with 3% FCS, 1 μM forskolin and NRG). Fibroblasts were cultured in DMEM with 10 % FCS.

Actin staining. Cells were fixed in 4% PFA, permeabilized with 0.2% Triton X-100 for 20 minutes and loaded with Alexa Fluor 594 Phalloidin for 30 minutes (Molecular Probes).

Nuclear and cell volume measurements by confocal sectioning. Sciatic nerves from E18 embryos were isolated in L-15 medium (Invitrogen) then immediately teased and then fixed in 4% PFA. DRG explants were isolated from E18 embryos and plated on cover slips coated with poly-L-lysine and laminin, in SF (without insulin and NRG) and fixed after 48h. The cells were loaded with Hoechst, fixed and stained for the Schwann cell specific marker, S-100 (Dako), using FITC-conjugated secondary. Confocal sections were taken and the volume determination from stacked images was performed using Volocity™ software. Due to the density of the Schwann cells in the sciatic nerve, it was not possible to measure the cell volume accurately in these samples. At least 100 cells were measured for each condition.

Image acquisition. All images were acquired with a Nikon Eclipse E800 in a BioRad Radiance 2100 Upright Multiphoton Confocal, at room temperature, with 40x (numerical aperture 1.3) and 60x (numerical aperture 1.4) objective lenses, equipped with LaserSharp 2000 acquisition software.

Nuclear and cell volume measurements by confocal. Nuclear and cell volume was calculated by loading the cells with Cell Tracker (Molecular Probes) and Hoechst 33342 according to the manufacturer's instructions. The cells were then fixed and sections were taken with a NikonE1000 confocal microscope. The volume determination from stacked images was performed using Volocity™ software. At least 100 cells were measured for each condition.

Shift experiments. Schwann cells (1.5 x 10^5 cells per dish) were plated in SF or SC on 6-cm-diameter dishes coated with PLL and laminin or fibronectin. Cells were passaged when the number of cells per dish was 3 x 10^5. 2 x 10^5 fibroblasts were plated on 9 cm dishes. Cell size and cell number were measured with a Coulter counter (Multisizer II; Beckman-Coulter). Protein content was determined with the BCA assay (Pierce).

Model. Cell-cycle times and growth rates were calculated for Schwann cells proliferating in SF and SC. Fibroblast cell cycle time and growth rate was calculated after six days in culture, when the cells had stabilised their size. Cell cycle times were calculated by using pulses of BrdU of increasing lengths of time. Growth rates were calculated by determining the increase in size/mass during the course of a cell-cycle. Assuming an instant change to a new linear growth rate and new rate of cell-cycle progression following a shift-up, the predicted cell size at the next division (S_1), was calculated using the equation

$$S_1 = (S_0 + gt)/2.^{14}$$

Where S_0 is the size of the cell before the shift, g is the new growth rate (e.g., the growth rate in SC for the Schwann cells and 10% serum for the fibroblasts) and t is the new division time. The size of a single cell *n* cycles after the shift is:

$$S_n = 1/2^n [S_0 + (2^n-1)gt].$$

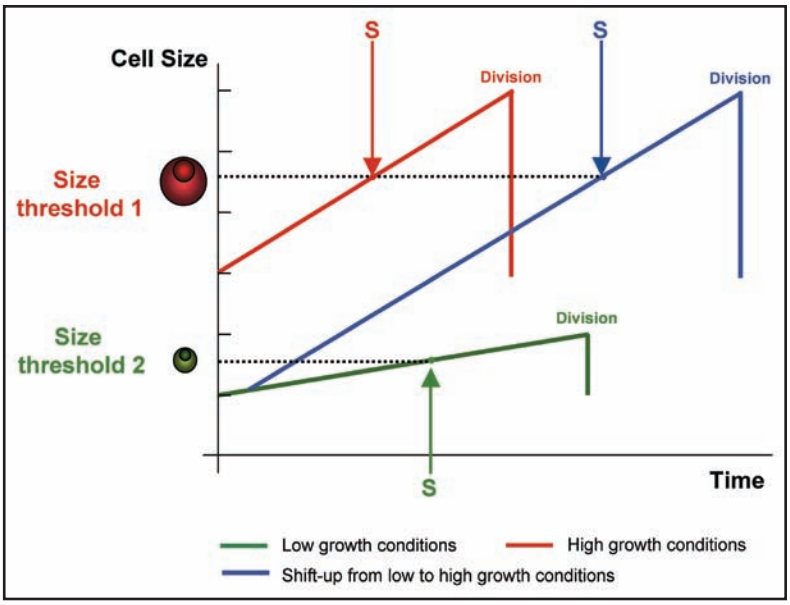

Figure 1. Consequences of an autonomous cell-size-sensing mechanism. If cells growing in conditions in which they are small (green) are transferred to conditions in which the size threshold to enter S-phase is much larger (red), entry into S phase should be delayed but the new cell size should be stabilised within one cell-cycle (blue). Conversely, following a shift-down cells should enter the first S-phase more rapidly.

RESULTS

In this study, we have explored how cell size is regulated in mammalian cells. To do this, we took advantage of the argument that the operation of an autonomous size-sensing mechanism should impose constraints on a population of cells when shifted between conditions of slow growth to conditions of faster growth and vice versa. For example, if cells are shifted up from a medium in which they are proliferating at a small size to a medium in which cells are proliferating at a large size (shift up), they should remain in G_1 until they reach the size required to start S-phase in the new culture conditions; as a consequence, the cells should show a delayed entry into the first S phase, and their size should stabilise within one cycle (Fig. 1). Conversely, if cells are shifted down from a medium in which they are large to a medium in which they are small, they should enter the first S phase earlier than in their previous condition and hence show a transient increase in proliferation.[3]

The rate of proliferation of Schwann cells is not determined by cell size. Primary rat Schwann cells can proliferate in culture indefinitely.[13] Depending on the culture medium, they exhibit large variations in size, making them a convenient model to explore how cell size is regulated.[6] As can be seen in Figure 2A, in steady-state conditions, the average size of Schwann cells proliferating in medium containing 3% foetal calf serum (SC) is 3-fold bigger than those proliferating in serum-free medium (SF). In contrast, whilst the cells in SC proliferate faster than those in SF, the difference is less than 2-fold (Fig. 2A).

For the shift up experiments, cells maintained in SF were transferred to SC. This is equivalent to transferring the cells from low to high levels of growth factors. If a cell-size checkpoint exists, the shifted cells should pause in G_1 until they reach the new larger size threshold required to enter into S-phase in SC; we would therefore expect to

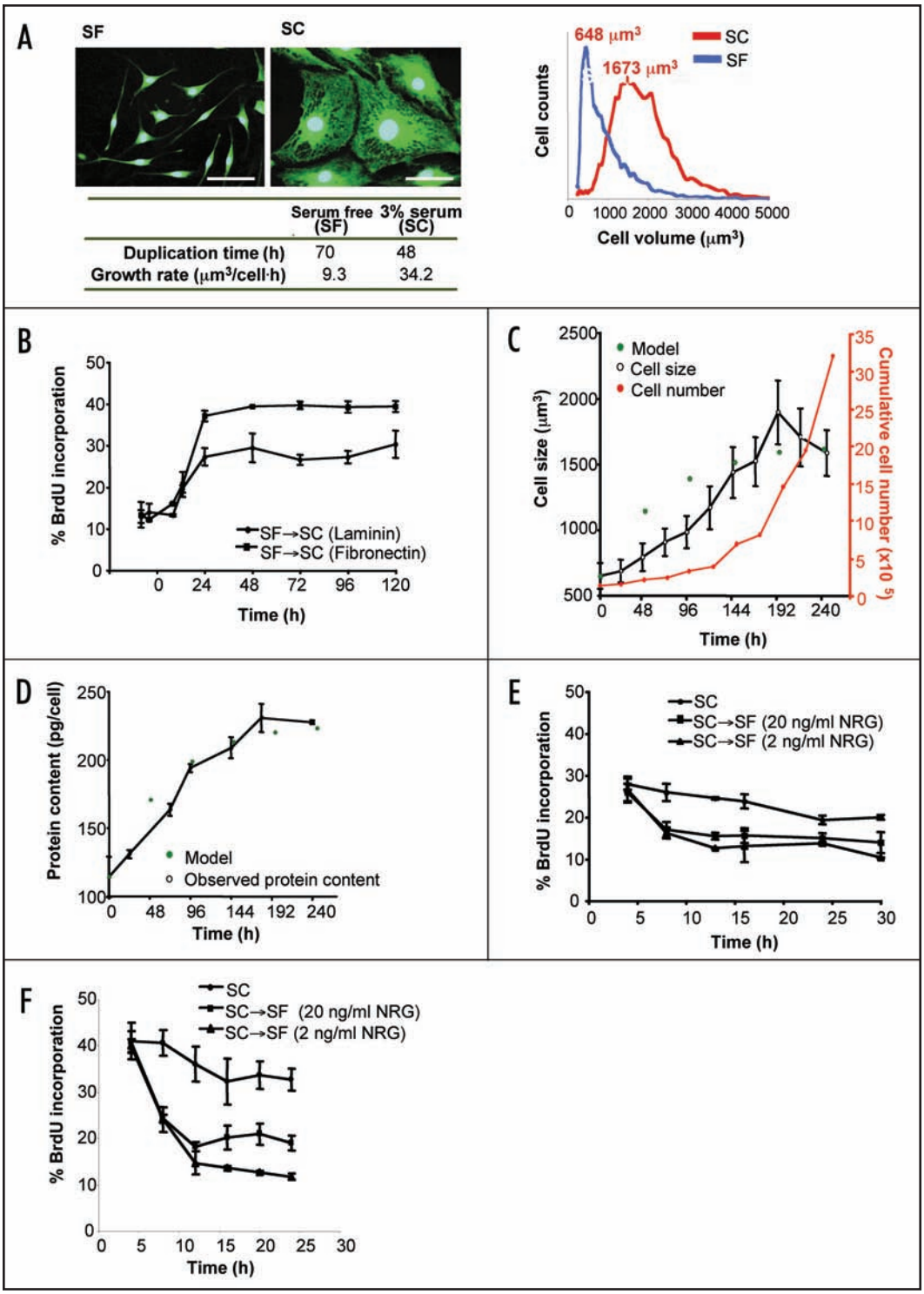

Figure 2. Schwann cell proliferation rate is independent of cell size. (A) Confocal images of cell-tracker-loaded Schwann cells and Coulter counter cell size measurements of Schwann cells proliferating in either SF or SC. Scale bar is 60 μm. (B) Pulses of BrdU (4 h) were used to measure the proliferation rate of Schwann cells when transferred from SF to SC on laminin or fibronectin. Mean ± s.d. are presented. (C) Average size, cumulative cell number and mass (D) of a population of Schwann cells shifted from SF to SC, measured in a Coulter counter (mean ± s.e.m.). Predicted size is shown in green calculated according to the equations described in the materials and methods. Pulses of BrdU (4h) were used to measure the proliferation rate after the shift from SC to SF, with cells plated onto laminin (E) or fibronectin (F).

see a decrease in the proliferation rate shortly after the shift and, if cell mass regulates proliferation rate, we might expect an increase in the proliferation rate, as the size of the cells progressively increases to their new steady-state size. However, if the rate of cell cycle proliferation is independent of cell size, then the cells should proliferate faster soon after the shift-up and maintain a constant rate of proliferation as the cell-size gradually increases. To test these models, we gave the cells short pulses of BrdU following the shift-up and then used immunostaining to determine the percentage of cells that had incorporated BrdU. We performed these experiments in two conditions: (i) our standard conditions, in which the cells were grown on laminin and the difference in proliferation rate is small and (ii) on fibronectin, where the difference in proliferation rate was greater. In both cases, the BrdU incorporation rate increased very soon after the shift, reaching a maximal constant level within 24h (Fig. 2B). This rapid increase in BrdU incorporation rate was mirrored by a rapid decrease in cell-cycle time, as measured by the increase in cell number following the shift-up (Fig. 2C). The cells required ~192 h to reach a stable size and protein content yet during this time they had a constant proliferative rate (Fig. 2B–D). These findings, coupled with the observation that it took more than three population doublings to reach the new steady-state size (Fig. 2C), demonstrate that cell size is not determining proliferation rate and that these cells do not appear to have a cell-size checkpoint. Instead, the rate of cell cycle progression appears to be controlled solely by the levels of extracellular factors.

Cell cycle progression rates are regulated by extracellular levels of mitogens. The steady-state size of Schwann cells maintained in SC should be larger than the size required for entry into S-phase in SF (see Fig. 1). Therefore, if cells in SC are transferred to SF

and the cells have a size checkpoint we should observe an acceleration of the proliferation rate shortly after the shift. Instead however, we observed a rapid decrease in the percentage of cells that incorporated BrdU to a lower constant value with the new proliferation rate dependent on the level of mitogen and the type of extracellular matrix (Fig. 2E and F). As in the case of the shift up, the down-shifted cells required several cycles to reach a stable size distribution (data not shown). Again, these results argue that the level of extracellular mitogens determines the proliferation rate without any input from the size of the cell.

Modelling cell size regulation in the absence of a cell size checkpoint. Our results suggest that changing the levels of extracellular growth factors and mitogens results in a rapid switch to new growth rates and cell cycle times. They imply that the rate of change in cell size and cell mass in different culture conditions and the time taken to reach a new steady-state size would solely depend on the differences in the growth and proliferation rates. This can be modelled mathematically, by calculating the predicted change in size and mass of cells following a shift-up, assuming an immediate change to a new linear rate of growth and a new cell cycle time. Thus, the predicted size of a single cell, one cycle after the shift (S_1) can be calculated according to the following expression:

$$S_1 = (S_0 + gt)/2,$$

where S_0 is the size of the cell before the shift (size of Schwann cells in SF), g is the steady-state growth rate calculated for Schwann cells grown in SC and t is the duplication time of cells in SC. A similar equation can be used to calculate the predicted protein content of the cells after the shift. Consistent with this model, the size and mass increases, observed in the Schwann cells following a shift-up, show a similar pattern to the predicted values (Figs. 2C and 2D).

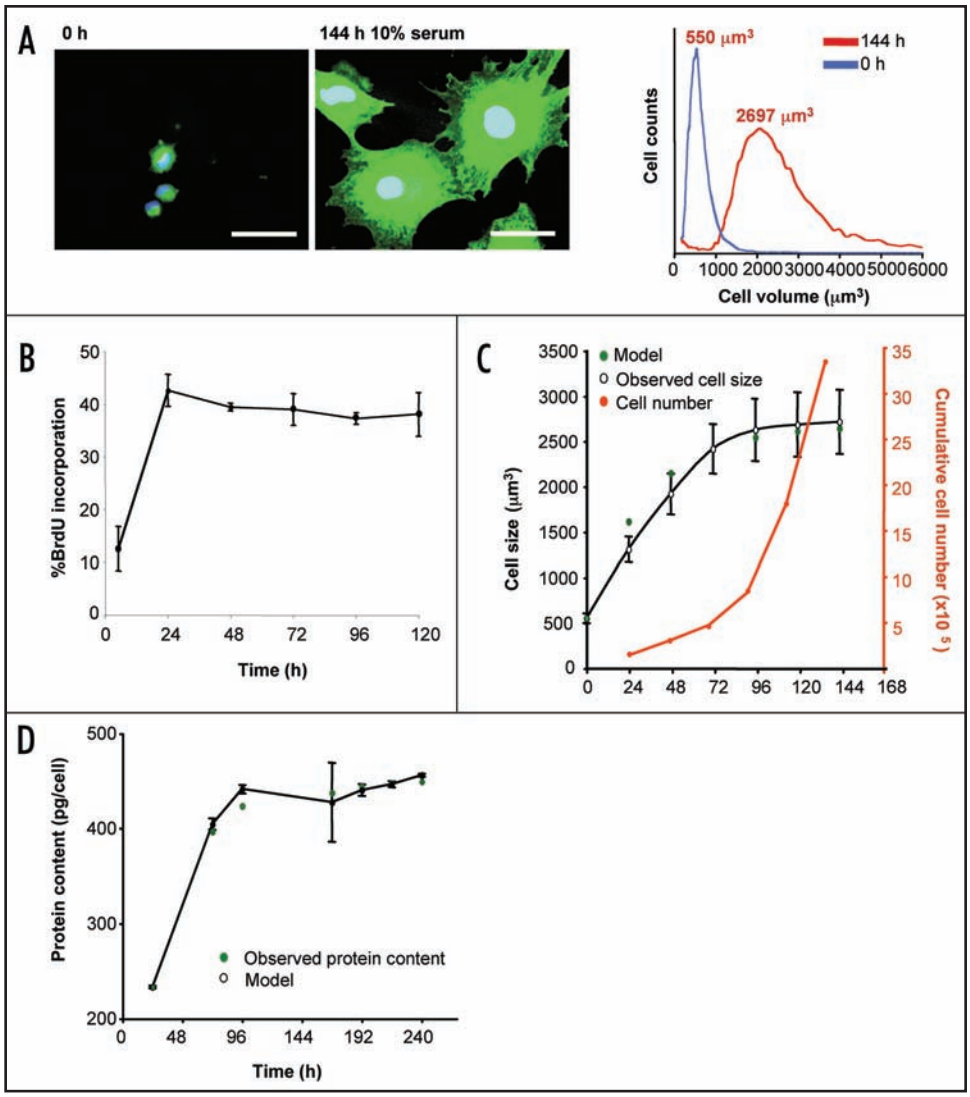

Figure 3. Absence of a cell-size checkpoint in fibroblasts. (A) Confocal images of cell-tracker-loaded fibroblasts and size (as measured in a Coulter counter) immediately after purification and after six days in culture in 10% FCS (144 h). (B) Pulses of BrdU (4 h) were used to measure the rate of proliferation after the shift from in vivo to in vitro conditions. (C) Average size, mass (D) and cumulative cell number of fibroblasts shifted from in vivo to in vitro conditions. The model values (green) were calculated using the same equation as for the Schwann cells with the growth rate (112 µm³/h), increase in mass (18.7 pg/h) and cell cycle time (24 h) calculated from the cells proliferating at their steady state size after 6 days in culture.

Fibroblast divide at a rate that is independent of cellular size. To test whether our findings with Schwann cells can be extended to other cell types we analysed fibroblasts that are present in the nerves from which we isolate the Schwann cells. We purified the fibroblasts to homogeneity by immunopanning and plated them in DMEM/10% FCS. We observed that the newly-purified fibroblasts were very small (550 µm³), approximately 5-fold smaller than their steady-state size when cultured in 10% FCS (2697 µm³) (Fig. 3A). As we were unable to culture these cells in the absence of serum, we studied their growth and cell-cycle progression immediately after isolation and considered the "switch-up" as the change from in vivo to 10% FCS in vitro.

Once plated in 10% FCS, more than 90% of the fibroblasts incorporated BrdU during the first 24 h. When we used short pulses of BrdU we found that the rate of cell cycle progression was very low immediately after purification but then increased rapidly, reaching a maximum, constant value within 24 h (Fig. 3B). The fibroblasts required ~120 h to reach a stable cell-size distribution (Fig. 3C) and the average protein content per cell followed a similar pattern (Fig. 3D). This period corresponded to approximately ~5 population doublings (Fig. 3C). These results strongly suggest that, for fibroblasts, like Schwann cells, the rate of cell-cycle progression is not controlled by cell size or mass. To test whether the fibroblasts conform to our model, we calculated the predicted increase in cell size and cell mass as for the Schwann cells assuming a linear growth rate and constant cell-cycle time. As shown in Figure 3C and D, the observed increase in cell size and cell mass fit the predicted

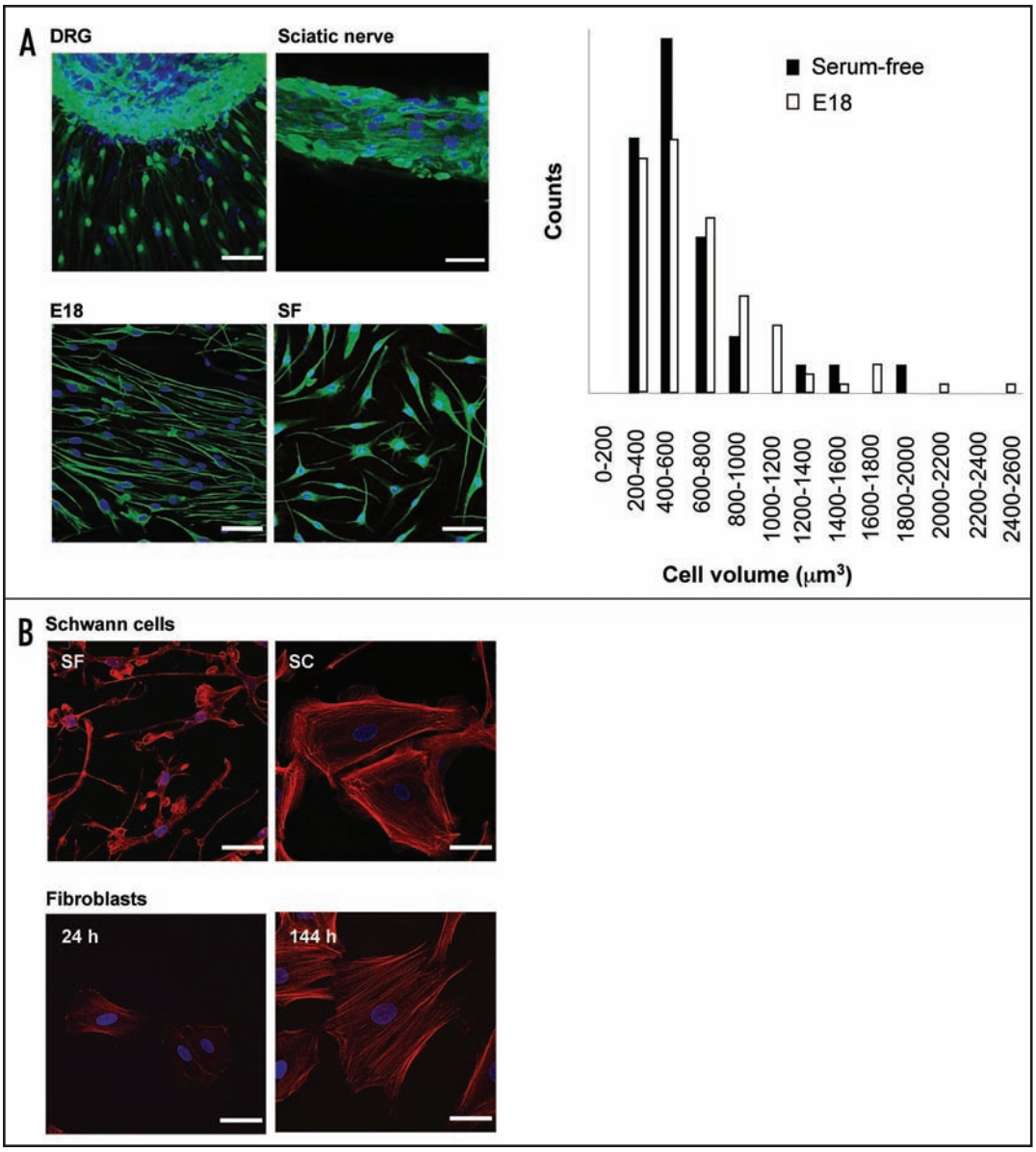

Figure 4. The size of Schwann cells in vivo. (A) DRG explants from E18 embryos were plated without mitogens or growth factors. 48 h later the cells were labelled with Hoechst and immunostained for the Schwann cell marker, S100 (Left hand panels). Teased sciatic nerves isolated from E18 embryos were similarly stained immediately after plating (upper right-hand panel). Cultured Schwann cells in SF were similarly labelled (bottom right-hand panel). Cell size and nuclear size was calculated from stacked confocal images (also see Table 1). Scale bar is 60 μm. (B) Phalloidin staining of Schwann cells in SF and SC and fibroblasts 24 h and 144 after plating in 10% FCS. Scale bar is 40 μm.

values. These results are consistent with a model in which cell size is solely determined by the level of extracellular factors, and not by an intrinsic cell-size sensor.

Physiological size of proliferating Schwann cells. To exclude the possibility that we were failing to observe a cell-size checkpoint in Schwann cells because we were working outside the normal size range at which the cells proliferate in vivo, we took two approaches. We determined the size of Schwann cells in teased sciatic nerves of E18 rat embryos, when Schwann cells are known to be proliferating and we measured the size of Schwann cells migrating from dorsal root ganglia (DRG) explants in the absence of any added extracellular growth factors or mitogens. Using stacked images of Schwann cells stained with the Schwann cell marker S100 and Hoechst dye, we determined the cell and nuclear size of the Schwann cells from the DRG explants and the size of the nuclei in the teased nerves. We confirmed the accuracy of this technique by comparing these measurements to those obtained from the Coulter-counter. In both teased nerves and explants, the size of the Schwann cells was very similar to the steady-state size of Schwann cells cultured in SF, suggesting that SF Schwann cells divide close to their physiological size (Fig. 4A and Table 1).

It has been postulated that changes in the nuclear/cytoplasmic (N/C) ratio may have a role in triggering the size checkpoint and/or controlling the proliferation rate of cells.[15] When we compared the N/C ratio of the cells in this study, we found that the larger the cells, the lower the N/C ratio (Table 1). However this change in ratio does not appear to affect the proliferation rate of the cells.

Cell size affects the behavior of mammalian cells. We have found that Schwann cells and fibroblasts proliferating in vitro in FCS are 300–500% bigger than their size in vivo. This massive increase in cell size is likely to have implications for the behaviour of these cells. We observed that as the cells got larger, they developed an altered morphology, appearing flatter and less polarised. Moreover, actin-staining demonstrated a change in cytoskeletal organisation. The cells in SF were elongated, with actin-staining strongly localised to multiple lamellipodia-like structures; in contrast, the cells in SC were flatter and did not extrude lamellipodia but instead had multiple actin-bundles around the periphery of the cell (Fig. 4B). These differences did not reflect short-term responses to FCS, as SF cells retained their distinct morphology and cytoskeletal organisation for at least 24 h after FCS addition. Likewise, the larger fibroblasts were flatter and had more prominent stress fibres than the smaller fibroblasts despite being cultured in the same medium (Fig. 4B).

DISCUSSION

The results of these experiments argue against the existence of a cell-size checkpoint. Instead, they suggest that Schwann cells and

Table 1 **Nuclear and cell size of Schwann cells and fibroblasts**

	Schwann Cells				Perineural Fibroblasts		Cell Lines		
	E18 Sciatic Nerve	E18 DRG	Serum-Free	3% Serum	In Vivo (p7)	10% Serum 144h	NIH3T3	Swiss3T3	Rat1
Nuclear volume (μm^3)	**277 ± 14**	**254 ± 12**	**333 ± 12**	**479 ± 19**	**272 ± 17**	**589 ± 20**	ND	ND	ND
Cell volume (μm^3)	ND	**697 ± 44**	648 ± 98 **598 ± 49**	1673 ± 244	550 ± 53.3	2697 ± 324	1540 ± 196	1785 ± 254	1427 ± 276
Nucleus/Cell ratio (%)	ND	**36**	51, **56**	29	49	22	ND	ND	ND

Bold type corresponds to stacked images obtained by confocal microscopy, whereas normal type corresponds to the values obtained using the Coulter counter. Values shown are mean ± s.e.m.

fibroblasts divide at a size that depends on how fast they are growing and how fast they are going through the cell-cycle, both of which vary according to the concentration of extracellular signals. In this model, switching between different levels of extracellular factors causes a rapid change to new rates of proliferation and growth, which can be determined independently by the levels of mitogens and growth factors. The differences in the growth rates and proliferation rates will then determine the number of divisions required to reach the new steady-state cell size distribution. We have determined these theoretical changes in cell size and mass and found that they closely mirror our experimental values. As expected, the model is able to accurately predict the number of cell cycles required to reach the new steady-state cell size.

This model is consistent with in vivo studies in *Drosophila* and mice where findings that disruption of various growth and cell-cycle regulatory pathways can result in altered cell size suggested that growth and cell proliferation can be independently regulated.[3,16-19] A recent study however, has claimed to provide evidence for yeast-like cell-size checkpoints in a variety of mammalian cells.[7] In the shift experiments reported in these studies, the differences in size were less than two-fold, which means that the cells could, in principle, adjust their size within one cycle, even without a cell-size checkpoint. Moreover, artificial extension of S phase by aphidicolin in these studies may have had additional effects on the cell-cycle. Other possible explanations for the differences between their studies and ours include that a gradual change in a size-checkpoint occurred in our experiments after the shift. We think this is unlikely as it should have resulted in a gradual change in proliferation rates, which we did not see. Our results however, do not preclude the possibility that there is a minimum size below which cells cannot enter the cell cycle. Indeed, it has been shown that lymphocytes undergoing autophagy following IL-3 removal, become very small and, upon IL-3 readdition, take several days to reenter the cell-cycle following a period of growth.[9] Whether this reflects the operation of a cell-size checkpoint will require further examination.

In cultured Schwann cells, growth and cell-cycle progression can be regulated independently by the extrinsic factors IGF1 and NRG, respectively.[11] This has allowed growth and cell-cycle progression to be studied separately in these cells. In many other cells, the separation of these two processes may be less clear, as many extracellular signals stimulate both growth and cycle progression. In many ways, this coregulation makes sense, in that it provides a simple mechanism to coordinately drive proliferation and growth and would thus be a useful strategy to produce large numbers of similarly sized cells. This coordination however, does not dictate the existence of a cell-size checkpoint. On the other hand, the use of separate extracellular signals to regulate these processes independently, allows an animal to produce cells of varying sizes by simply changing the concentrations of these factors.

We have observed that commonly used culture conditions, using FCS, can produce proliferating cells that are 3–5 fold larger than they normally are in vivo. The reason they are so big is because factors in FCS increase growth rate much more than they increase the rate of cell-cycle progression. In other words, FCS is a much more potent growth factor than it is a mitogen. As most cells are cultured in serum, it is likely that they are operating at an abnormally higher growth rate than is seen in vivo and are functioning at an abnormally large size. This is consistent with the large size of fibroblast cell lines commonly studied in culture (Table 1) and is likely to be the case for many cells cultured in FCS. We have shown that the increase in cell size is associated with changes in cell morphology, but it is likely that there are many other effects on the physiology of the cells, as the differences in size reflect changes in the protein synthesis and metabolic rate. Indeed, we consider it likely that this drastic change to the metabolism of the cell contributes to the general "culture-shock" phenomenon seen when cells are removed from an animal and placed into culture.

References

1. Fantes P, Nurse P. Control of cell size at division in fission yeast by a growth-modulated size control over nuclear division. Exp Cell Res 1977; 107:377-86.
2. Johnston GC, Pringle JR, Hartwell LH. Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. Exp Cell Res 1977; 105:79-98.
3. Jorgensen P, Tyers M. How cells coordinate growth and division. Curr Biol 2004; 14: R1014-27.
4. Grewal SS, Edgar BA. Controlling cell division in yeast and animals: Does size matter? J Biol 2003; 2:5.
5. Wells WA. Does size matter? J Cell Biol 2002; 158:1156-9.
6. Conlon I, Raff M. Differences in the way a mammalian cell and yeast cells coordinate cell growth and cell-cycle progression. J Biol 2003; 2:7.
7. Dolznig H, Grebien F, Sauer T, Beug H, Mullner EW. Evidence for a size-sensing mechanism in animal cells. Nat Cell Biol 2004; 6:899-905.
8. Brooks RF, Shields R. Cell growth, cell division and cell size homeostasis in Swiss 3T3 cells. Exp Cell Res 1985; 156:1-6.
9. Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, Thompson CB. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005; 120:237-48.
10. Taton M, Dumont JE. Dissociation of the stimuli for cell hypertrophy and cell division in the dog thyrocyte: Insulin promotes protein accumulation while TSH triggers DNA synthesis. Exp Cell Res 1995; 221:530-3.
11. Conlon IJ, Dunn GA, Mudge AW, Raff MC. Extracellular control of cell size. Nat Cell Biol 2001; 3:918-21.
12. Su TT, O'Farrell PH. Size control: Cell proliferation does not equal growth. Curr Biol 1998; 8:R687-9.
13. Mathon NF, Malcolm DS, Harrisingh MC, Cheng L, Lloyd AC. Lack of replicative senescence in normal rodent glia. Science 2001; 291:872-5.
14. Brooks RF. Random transitions and cell cycle control. Prog Clin Biol Res 1981; 66(Pt A):593-601.
15. Umen JG. The elusive sizer. Curr Opin Cell Biol 2005; 17:435-41.
16. Lawlor MA, Mora A, Ashby PR, Williams MR, Murray-Tait V, Malone L, Prescott AR, Lucocq JM, Alessi DR. Essential role of *PDK1* in regulating cell size and development in mice. Embo J 2002; 21:3728-38.

17. Montagne J, Stewart MJ, Stocker H, Hafen E, Kozma SC, Thomas G. *Drosophila* S6 kinase: A regulator of cell size. Science 1999; 285:2126-9.
18. Neufeld TP, de la Cruz AF, Johnston LA, Edgar BA. Coordination of growth and cell division in the *Drosophila* wing. Cell 1998; 93:1183-93.
19. Shima H, Pende M, Chen Y, Fumagalli S, Thomas G, Kozma SC. Disruption of the *p70*(*s6k*)/*p85*(*s6k*) gene reveals a small mouse phenotype and a new functional S6 kinase. Embo J 1998; 17:6649-59.