Developing Dictyostelium cells form evenly sized groups of \(2 \times 10^4\) cells. A secreted 450-kDa protein complex called counting factor (CF) regulates group size by repressing cell-cell adhesion and myosin polymerization and by increasing cAMP-stimulated cAMP production, actin polymerization, and cell motility. We find that CF regulates group size in part by repressing internal glucose levels. Transformants lacking bioactive CF and wild-type cells with extracellular CF depleted by antibodies have high glucose levels, whereas transformants oversecreting CF have low glucose levels. A component of CF, countin, affects group size in a manner similar to CF, and a 1-min exposure of cells to countin decreases glucose levels. Adding 1 mM exogenous glucose negates the effect of high levels of extracellular CF on group size and mimics the effect of depleting CF on glucose levels, cell-cell adhesion, cAMP pulse size, actin polymerization, myosin assembly, and motility. These results suggest that glucose is a downstream component in part of the CF signaling pathway and may be relevant to the observed role of the insulin pathway in tissue size regulation in higher eukaryotes.

Little is known about how a multicellular organism regulates the size of its tissues during development. An example of size regulation occurs in the simple eukaryote Dictyostelium discoideum, which during development forms groups of \(2 \times 10^4\) cells. Dictyostelium grows on soil surfaces and feeds on bacteria as an individual amoeba when the food source is ample (for review see Refs. 1 and 2). The cells eventually overgrow their food supply, and a cell signals that it is starving by secreting a cell density-sensing factor called conditioned medium factor (3–5).

As more and more cells starve, the local conditioned medium factor concentration reaches a threshold level. This allows cells to aggregate using relayed pulses of cAMP as a chemoattractant (6, 7). The cells form dendritic aggregation streams (for review see Refs. 2 and 8). The cAMP pulses also regulate the expression of many genes specifically expressed during early development (for review see Ref. 9). A rapid cGMP pulse is also generated in response to a CAMP pulse (9, 10). The aggregating cells produce a fruiting body consisting of a spore mass held atop a rigid stem of stalk cells. When a fruiting body is too small, the spore mass will be too close to the ground for optimal spore dispersal. If a spore mass is too big, it will slide down the stalk or the fruiting body will fall over. Therefore, the formation of an optimal size spore mass and stalk is of utmost importance (for review see Ref. 11).

One of the ways in which an upper limit to fruiting body size is established is by breaking up large aggregation streams into groups of \(2 \times 10^4\) cells, with each group developing into a fruiting body (12).

A secreted protein complex regulates stream breakup and thus group size. Wild-type cells secrete this protein complex, named counting factor (CF), at a moderate level. Disruption of the smlA gene leads to the oversecretion of CF, resulting in the formation of much smaller groups than wild type (13). In smlA− cells, the streams are severely fragmented, thereby forming large numbers of tiny fruiting bodies. The addition of purified CF to wild-type cells also decreases group size. CF seems to consist of at least five polypeptides, forming a complex of about 450 kDa. Two of these polypeptides are countin and CF50. When the countin or cf50 genes are disrupted, the cells secrete very little detectable CF activity (14, 15). countin+ and cf50− cells form abnormally large groups, leading to the formation of large fruiting bodies that readily collapse. Streams of countin− or cf50− cells seldom break, thus forming few but huge fruiting bodies (14, 16).

We used Monte Carlo simulations of streaming cells to model and test the effect of various parameters on stream breakup. The simulations indicated that group size increases with the ratio of adhesion force to motility force (16, 17). When both adhesion and motility are controlled by a secreted factor such as CF, more precise group control can be achieved. One of the major adhesion molecules utilized during early development is gp24 (18). gp24 is down-regulated by CF, and when anti-gp24 antibodies were added to the cells to mimic this effect the group size decreased (16). CF also decreases group size by increasing the amount of polymerized actin (F-actin) and decreasing the amount of polymerized myosin II, which in turn increases the motility of cells (17). In addition, CF down-regulates the cAMP-induced cGMP pulse and up-regulates the cAMP-induced cAMP pulse (19). These observations suggested that CF regulates the cAMP-induced cGMP and cAMP pulses and that these may in turn mediate the effect of CF on adhesion and motility.

Garrod and Ashworth (20) showed that wild-type cells grown with a high concentration (86 mM) of glucose formed 2.6 times larger fruiting bodies than control cells. In addition, Kishi et al. (21) found that 1 mM glucose can trigger Dictyostelium spores to dephosphorylate actin, a key step in the germination pathway, indicating that relatively low levels of glucose can act as a signal in Dictyostelium. In growing and developing cells the internal glucose concentration is ~2.4 mM but tends to vary depending on growth conditions and cell density (22, 23). How-
ever, the concentrations of subsequent products of glycolysis such as glucose-6-phosphate are very constant (22, 23). This appears to be due to the fact that the \( K_m \) of Dictyostelium glucokinase for glucose is 0.12 mM (24). Thus the internal glucose concentration in the cells is sufficient to saturate the enzyme, suggesting that the rate of glycolysis would be constant even when the levels of glucose are very different in cells. Together, these observations suggested the possibility that there might be a relationship between glucose levels and group size.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—** *D. discoideum* Ax4 wild-type, *smlA*-, and *countin*—cells were grown in HL5 medium (with maltose as the carbohydrate source) and starved in PBM (20 mM KH₂PO₄, 10 mM CaCl₂, 1 mM MgCl₂, pH 6.1) as described previously (13, 14). When indicated, the cells were starved in PBM containing 200 ng/ml recombinant countin, 200 ng/ml recombinant CF50, or a 1:1500 dilution of affinity-purified anti-recombinant countin antibodies (final concentration, 0.7 μg/ml IgG) (15, 25) or PBM containing 1 mM glucose (Sigma). The cells were starved on filters according to Tang et al. (19). For development on glucose agar, the cells were plated onto 1.5% agar plates containing 0, 1, or 10 mM glucose in PBM. The cells were starved in the presence of cAMP-specific phosphodiesterase or cAMP pulses according to Tang et al. (19).

**Glucose Assay—** Glucose levels were measured with the Trinder glucose assay kit (Sigma) with a minor modification to the user’s manual; we have found that it is very important to slowly stir the glucose assay reagent in a bottle wrapped with aluminum foil until the solution is completely dissolved. 10 μl of cells starving in shaking cultures were collected at 0, 2, 4, and 6 h of development by centrifugation at 400 × g for 3 min. The pellets were resuspended in 1 ml of PBM, transferred to Eppendorf tubes and centrifuged at 12,000 × g for 3 min. The pellets were lysed by freezing at −70 °C for 2 or more h. The pellets were thawed and 200 μl of distilled water, or PBM was added to the tube. The lysates were clarified by centrifugation at 12,000 × g for 3 min, and 100 μl of the supernatants was mixed with 3 μl of Trinder reagent in test tubes. To measure extracellular glucose, the cells were starved in shaking culture. Conditioned media were collected at 2, 4, and 6 h, concentrated 10-fold by lyophilizing overnight, and resuspended in distilled water. After incubating for 18 min at room temperature, the absorbances were measured at 505 nm. At the same time, 10 μl of the supernatant was mixed with 40 μl of distilled water, and 10 μl of this was used for a Bio-Rad protein assay. The percentages of change in glucose levels are given as the means ± S.E. from at least three independent assays.

**Western Blot Analysis—** The cells were grown in shaking culture in the presence or absence of 1 mM glucose for 16 h and starved in shaking culture, in the presence or absence of 1 mM glucose. After 4 and 6 h of starvation, conditioned medium was collected, concentrated up to 40-fold using 10 kDa MWCO Microcon spin filters (Millipore, Hercules, CA), and stained for countin according to Brock and Gomer (14). At 0 and 6 h of starvation, the cell pellets were collected and stained for gp24 expression according to Roisin-Bouffay et al. (16).

**Determining the Effects of Sugars on Development—** The cells were starved on filters soaked with PBM. After 2 h, the filters were transferred to pads soaked with PBM, 200 ng/ml recombinant countin (25), 200 ng/ml recombinant CF50 (15), recombinant countin with 1 mM glucose, or recombinant CF50 with 1 mM glucose. For other assays, the filters were transferred at 2 h after starvation onto support pads soaked with PBM. The sugars listed in Table I. Aggregates and fruiting bodies were examined and counted at 14 and 24 h, respectively.

**Adhesion Assays—** The cells were starved on filters for 1 h. The filters were then transferred to pads soaked with 1 or 10 mM glucose, 3-O-methylglucose, mannose, isocitrate, or PBM. At 2, 4, and 6 h after starvation, adhesion was measured as described previously (16). To measure the adhesion of vegetative cells, *smlA*+ cells were grown overnight in HL5 in the presence or absence of 1 mM glucose and washed once in Bonner’s salt solution (10 mM NaCl, 10 mM KCl, 2 mM CaCl₂), and the adhesion assay was performed as described using Bonner’s salt solution instead of PBM.

**Motility Assay, Actin Polymerization, Myosin Assembly, and Computer Simulations—** Cells in mid-log phase were diluted in PBM to 3 × 10⁵ cells/ml and 300 μl was placed in each well of an eight-well glass slide. At 1 h after starvation, glucose was added to final concentrations of 0, 1, or 10 mM. At 6 h of starvation, motility was measured by videomicroscopy as described in Yuen et al. (4). Actin polymerization and myosin assembly were assayed as described previously (17). The computer simulations used the program described by Roisin-Bouffay et al. (16).

**cAMP and cGMP Production Assays—** The cGMP production in response to a stimulus with 1 μM extracellular cAMP and the cAMP analog in response to 10 μM of the cAMP analog 2′-deoxy-cAMP were measured according to Tang et al. (19).

**RESULTS**

**CF Negatively Regulates Internal Glucose—** Because glucose might be involved in fruiting body size regulation (20), we measured the levels of internal glucose in *smlA*−, wild-type, and *countin*− cells. During both vegetative growth and development in submerged shaking culture, *smlA*− cells had the lowest levels of internal glucose, with *countin*− cells having the highest (Fig. 1A). Converting our data, we obtained −3 mM glucose in packed wild-type cells, indicating that our observations are consistent with the previously observed value of 2.4 mM (22, 23).

Because internal glucose levels were different in these cell lines, we measured the levels of glucose when recombinant countin and anti-countin antibodies were added to wild-type cells. This allowed us to examine the effect of CF on glucose in a single clone of cells grown in the same flask. Recombinant countin appears to have the same activity as purified CF and causes the formation of small fruiting bodies (25). The addition of recombinant countin at 1 h after starvation decreased the amount of internal glucose in wild-type cells at 6 h (Fig. 1B). We previously found that addition of anti-countin antibodies to starving cells depletes CF and causes the formation of large fruiting bodies (14). As shown in Fig. 1B, anti-countin antibodies increased glucose levels in wild-type cells. Taken together, the data suggest that CF negatively regulates glucose levels. Recombinant countin affects actin and myosin polymerization within 60 s (25). To determine whether CF affects glucose levels with a similar rapidity, we treated wild-type cells with recombinant countin for 1 min, collected them by centrifugation, and froze them. The cells were thus exposed to recombinant countin for more than 5 min. Exposure of cells to 2, 20, or 200 ng/ml of recombinant countin for this time significantly decreased levels of internal glucose (Fig. 1C). We have found that, compared with 100–200 ng/ml, levels of recombinant countin above −1000 ng/ml are much less effective at decreasing group size (25). In rough agreement with this, 2000 ng/ml of recombinant countin had little effect on glucose levels (Fig. 1C).

**Exogenous Glucose Increases Internal Glucose—** To determine whether exogenous glucose would increase internal glucose levels, the cells were starved in shaking culture with 0, 1, or 10 mM glucose. Starving wild-type cells in the presence of 1 or 10 mM glucose increased their internal glucose levels by −30%, to the point where the glucose levels were comparable with those seen in *countin*− cells. This indicates that glucose was transported into the cells and allowed wild-type cells to mimic *countin*− cells. This observation that 1 and 10 mM glucose raised the internal level about the same percentage suggested that there is a limit to how much glucose can enter cells. To verify that CF is regulating glucose, not glucose regulating the secretion of CF, we examined the effect of adding 1 mM glucose on the amount of countin in starvation medium conditioned for 4 or 6 h. The addition of glucose did not affect the amount of countin accumulated in the conditioned starvation medium as assayed by Western blots (Fig. 1D). The above results suggest that glucose added to starving cells increases internal glucose levels without affecting CF secretion.

**Exogenous Glucose Increases Group Size—** To determine whether increasing glucose increases group size, wild-type cells were starved with low concentrations of glucose (1 and 10 mM). With 1 mM glucose, there was no significant change in the...
A Factor Regulates Glucose to Control Group Size

Fig. 1. CF affects the levels of internal glucose. A, countin- cells were starved by shaking in PBM and harvested at the times indicated, and the level of internal glucose was measured. The values are the means ± S.E. The data shown are from nine (countin-), four (wild type), and three (smlA-) independent assays. B, wild-type cells were starved with recombinant countin or anti-countin antibodies (to deplete extracellular CF) in shaking culture. The amount of glucose in recombinant countin-treated cells was different from control with p < 0.01 (paired t test) from nine independent assays, and the amount of glucose in cells treated with anti-countin antibodies was different from control with p < 0.05 (t test) from five independent assays. C, wild-type cells were starved by shaking in PBM and exposed to recombinant countin for 1 min. The amounts of glucose in cells treated with 200, 20, and 2 ng/ml recombinant countin were different from control with p < 0.01, 0.06, and 0.1 (paired t test) from five, three, and three independent assays, respectively. D, wild-type cells were starved in the presence or absence of 1 mM glucose, and the conditioned media were collected at the times indicated. Concentrated conditioned media were separated by SDS-PAGE, and the protein blots were stained with anti-countin antibodies.

Timing of developmental processes (i.e., ripples in the field of cells, aggregation, streams, mounds, tipped mounds, finger formation, Mexican hat stage, and culmination). The addition of 10 mM glucose, however, delayed development by 2–3 h. We found that the addition of glucose increased the group size at low density (≈5 × 10^3 cells/cm^2 spotted on filters) (Fig. 2, A–C), at high density (≈10^4 cells/cm^2 spotted on filter pads) (Fig. 2, D–F), and on glucose agar plates (Fig. 2, G–I). The addition of 1 mM glucose increased the aggregate sizes and reduced the number of aggregates by 30 ± 3% (paired t test: p < 0.05, n = 3). Glucose concentrations below 0.3 mM had no discernible effect on group size. Glucose analogs and other products of glycolysis did not significantly affect group size, suggesting that not all sugars have this ability (Table I).

To determine whether cells might use secreted glucose as a signal to regulate group size, conditioned medium was collected from starving cells at timed intervals and concentrated by lyophilization. Glucose levels in the concentrated samples were then measured. At 6 h after starvation, smlA- conditioned medium had very low levels of glucose (Fig. 2J). The similar levels of glucose in wild-type and countin- conditioned media suggested that there is a limit to how much glucose can be secreted into the environment, and the observation that conditioned media had an ~600-fold lower glucose concentration than inside cells suggested that during development in shaking culture extracellular glucose concentrations are lower than the intracellular concentrations and the concentrations needed to affect group size.

To test the hypothesis that glucose may be a possible downstream regulator of CF, we determined whether glucose could suppress the effects of exogenous CF. We treated wild-type cells with two different components of CF (countin and CF50) alone or in combination with 1 mM glucose. We found that recombinant countin and recombinant CF50 increased group number as previously observed (15, 25). When recombinant countin was added in the presence of 1 mM glucose, the number of groups decreased compared with the number formed when cells were treated with recombinant countin alone (Fig. 3, A–D). This suggests that the effect of recombinant countin was partially rescued by the addition of 1 mM glucose. Similar results were obtained using recombinant CF50 (Fig. 3, E–G).

Exogenous Glucose Increases Cell-Cell Adhesion and gp-24 Expression—Compared with wild-type, countin- cells have a high cell-cell adhesion and smlA- cells have a low cell-cell adhesion during the first 4 h of development (16). Adding 1 mM glucose increased cell-cell adhesion in smlA- cells by 26.1 ± 3.8% at 2 h and 11.6 ± 0.9% at 4 h. We also found that glucose increased cell-cell adhesion in wild-type cells by 26 ± 7% at 4 h and 63 ± 22% at 6 h (means ± S.E.; n = 3). A randomly chosen subset of the products of glycolysis and glucose analogs that did not affect group size in wild-type cells also did not alter cell-cell adhesion of smlA- cells (Table II). smlA- cells that were grown with 1 mM glucose overnight had significantly higher cell-cell adhesion than cells that were grown without any glucose (Fig. 4A). We previously found that CF represses levels of the cell-cell adhesion molecule gp24 (16). The addition of 1 mM glucose at the time of starvation slightly increased the expression of gp24 6 h later in smlA- cells (Fig. 4B). In addition, smlA- cells grown overnight with 1 mM glucose had elevated levels of gp24 expression in vegetative cells (Fig. 4B). The cells grown over-
night and starved with 1 mM glucose also had a higher level of gp24 expression 6 h after starvation compared with control cells (Fig. 4B).

Besides decreasing adhesion, CF increases motility by increasing F-actin and decreasing the level of polymerized myosin II (17). The addition of 1 mM glucose decreased motility in wild-type cells in low density submerged culture (Fig. 5A). Wild-type cells starved on filter pads with glucose also had lower motility than control cells (data not shown). To determine whether glucose affects the level of F-actin, we prepared crude cytoskeletons from developing cells stimulated with extracellular cAMP. As previously observed, the level of F-actin peaked after 2 hours 4 hours 6 hours stimulation (Fig. 5B). A second peak of F-actin then appeared at 60 s. In the presence of 1 mM glucose, there were generally lower levels of F-actin, with the exception of a transient increase in F-actin at 5 s after cAMP stimulation (Fig. 5B). There was no discernible effect of glucose...
on total actin levels. To determine the effect of glucose on the level of myosin assembly, cytoskeleton preparations were separated on SDS/gel electrophoresis. Untreated cells had a pattern of myosin assembly similar to what was previously observed for wild-type cells (17), whereas cells grown and starved in the presence of 1 mM glucose had higher basal levels of myosin assembly (Fig. 5C). When cells were starved but not grown in the presence of glucose, there was no discernible effect on the levels of polymerized myosin. There was no discernible effect of glucose on total myosin levels (Fig. 5C). These results suggested that glucose increases group size by up-regulating cell-cell adhesion and down-regulating motility and that glucose affects actin and myosin polymerization.

**Exogenous Glucose Reduces cAMP Pulse Size**—CF regulates the cAMP signal transduction pathway (19). To determine the role of glucose in the CF signal transduction pathway, we tested the effect of cAMP pulses on the levels of internal glucose and vice versa. Cells treated with phosphodiesterase or cAMP had similar glucose levels compared with control cells (data not shown). This suggested that cAMP pulse size did not have any significant effect on glucose. However, cAMP-induced cAMP production was significantly reduced by the addition of 1 mM glucose (Fig. 6A). The cAMP-induced cGMP pulse was also examined in the presence or absence of 1 mM glucose (Fig. 6B). In wild-type cells, the cAMP-induced cGMP pulse peaks around 10 s (27). When glucose was added, the peak was delayed to around 30 s, suggesting that glucose affects the cAMP-induced cGMP pulse. In both the cAMP and cGMP assays, cells that were not treated with glucose had responses similar to those previously observed (19).

**DISCUSSION**

CF decreases group size in *Dictyostelium* (13, 14, 16, 17, 19). In this report, we find that CF decreases internal glucose levels and that low glucose levels cause a decreased group size.

We observed that there are differences in glucose levels in *smlA*−, wild-type, and *countin*− cells and that these differences can be observed in mid-log phase vegetative cells (Fig. 1A). However, these cells appeared to have indistinguishable growth rates, suggesting that they are using energy at the same rate. One possible explanation for this could be that although the glucose levels are different, the flux of glucose through the glycolytic pathway is the same in these cells. This is consistent with previous observations that although glucose levels tend to vary depending on culture conditions, the levels of intermediates in the glycolysis pathway appears to be invariant (22, 23). The ability of countin to affect glucose levels within 60 s suggests the existence of a fast signal transduction

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2 R. Ammann and R. H. Gomer, unpublished data.
pathway. How the glucose levels are changed so quickly remains to be elucidated.

Adding recombinant countin or depleting countin at the time of starvation alters internal glucose levels during later development. However, the degree of change is not as great as what we see in the different cell lines (Fig. 1, A and B). One of the possible explanations for this is that smIA- and countin- cells start development with different concentrations of glucose compared with wild-type cells. We have found that components of CF are secreted and affect cells during vegetative growth (15, 25). Therefore, smIA- and countin- cells are exposed to different levels of CF even before starvation, which may explain the different glucose levels in vegetative cells. Extracellular glucose levels in smIA-, wild-type, and countin- cells starved at 5 × 10^8 cells/ml are less than 6 μM. Assuming that starving cells are ~2 × 10^8 cells/ml in the stream and ignoring diffusion out of the stream or reuptake by the cells, the extracellular glucose concentration would then be ~0.24 mM, less than the minimal concentration needed to affect group size or motility. This then suggests that secreted glucose does not play a significant role in group size regulation.

Exposure of starving cells to 1 or 10 mM glucose caused a clear increase in group size (Fig. 2). Group size can be affected by territory size, stream breakup, or mound breakup. We observed that, like CF, glucose affected stream breakup rather than altering territory size or mound breakup. Glucose partially negated the effects of the addition of key components of the CF complex, countin and CF50, suggesting that glucose may be a downstream effector of the CF signal transduction pathway (Fig. 3). Glucose affected two main downstream targets of CF, cell-cell adhesion and motility. Increasing glucose or decreasing CF both increase gp24 levels, adhesion, and myosin polymerization and decrease actin polymerization and motility, suggesting that glucose affects group size by a pathway qualitatively similar to that used by CF. CF increases the size of the cAMP-induced cAMP pulse (19), and the addition of glucose decreased the cAMP pulse size (Fig. 5A). Together, these observations suggest that glucose may affect and be part of the CF signal transduction pathway. However, at this point, we do not know whether the effector is glucose per se or one of its metabolites.

Glucose increased cell-cell adhesion by 11.6% and decreased motility by 27.8%. We previously observed that small changes in adhesion and motility can affect group size (16, 17). Using computer simulations to model stream breakup, we observed that although changing adhesion alone or motility alone by these amounts only slightly increased group size (Fig. 7), changing both adhesion and motility by these amounts increased group size by roughly 30%, the amount observed with glucose.

The only significant difference we observed between countin- cells and cells exposed to 1 mM glucose was in the cAMP-induced cGMP pulse (Ref. 19 and Fig. 5B). countin- cells have a large and prolonged cGMP pulse, whereas high glucose causes cells to have a prolonged pulse. This suggests that CF affects a pathway in addition to glucose.

It is unclear why the levels of glucose or one of its metabolites appear to be part of a signal transduction pathway. In Escherichia coli, glucose inhibits CAMP synthesis, and low glucose levels increase the levels of cAMP (28, 29). Our observations that glucose inhibits the cAMP pulse are thus similar to those observed in E. coli. Having found that low cAMP pulses cause motility to decrease (17), our working hypothesis is that the countin-glucose-adhesion/motility pathway may have evolved from a chemotaxis mechanism used by an ancestral motile unicellular eukaryote. When this cell was in an area with low nutrients, as indicated by low internal glucose levels, we hypothesize that it would try to move by increasing motility and decreasing adhesion. However, when it found a food source and nutrient levels and possibly internal glucose levels increased, the cell would try to stay at the source by decreasing motility and increasing adhesion. We believe that this simple mechanism may have, during evolution, become modulated by the CF signal to regulate group size. We do not know the molecular mechanism whereby glucose affects the cAMP-induced cAMP pulse, adhesion, or motility. In other systems, glucose appears to affect adenylyl cyclase and Akt/PKB (30, 31). Because these regulate the cAMP pulse and motility, respectively, in Dictyostelium (32, 33), one possibility is that these processes may be regulated by glucose.

In mammalian cells and Caenorhabditis elegans, the insulin
or insulin-like growth factor signal transduction pathway plays an important role in cell and/or tissue growth by regulating protein synthesis (for review see Ref. 34). In Drosophila, body size decreases when mutations occur in genes for the insulin receptor, CHICO (a homolog of the insulin receptor substrate), phosphatidylinositol-3-OH kinase, and S6 kinase (35–38). When phosphatidylinositol-3-OH kinase is overexpressed in the responsive primordia and the flies are grown on rich medium, an increase in the size of wings and eyes can be observed. Insulin regulates the level and uptake of glucose in higher eukaryotes (39). Nutrients can regulate cell growth and proliferation through the mammalian target of the rapamycin (mTOR) pathway (for review see Ref. 40). Protein synthesis is also controlled by signaling through mTOR (41, 42). Insulin regulates the level and uptake of glucose in higher eukaryotes (39). Nutrients can regulate cell growth and proliferation through the mammalian target of the rapamycin (mTOR) pathway (for review see Ref. 40). Protein synthesis is also controlled by signaling through mTOR (41, 42). Insulin activates eukaryotic initiation factors through the mTOR signaling pathway, and 1–5 mM glucose promotes this activity of insulin (43). Because insulin elevates cytosolic glucose levels, this suggests that insulin activates mTOR partially by increasing glucose levels. Thus an intriguing possibility is that secreted factors regulate glucose levels to regulate tissue size in higher eukaryotes.

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