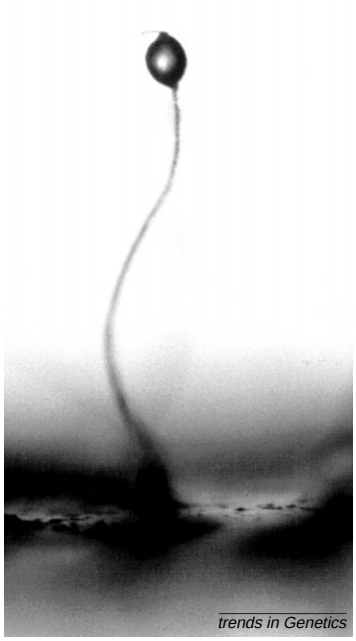
# Just the right size
## cell counting in *Dictyostelium*

**The regulation of tissue and organism size plays an essential, but poorly understood, role in multicellular development. Genes have been identified that affect body and organ size in a number of animals. Two recently identified genes, *smlA* and *countin*, are required for the proper function of a cell-counting mechanism that regulates organism size in the eukaryotic microorganism *Dictyostelium discoideum*. The discovery of this process now allows the study of size regulation in a simple multicellular system.**

Decades of study of the processes that control multicellular development have yielded a wealth of information. Yet one of the most interesting questions remains largely unanswered: how are the sizes of an organism and its component tissues regulated? The possibility that the mechanisms that govern organism size can be changed has spawned an entire film genre in which giant insects terrorize unsuspecting teenagers and overgrown lizards attack crowded cities. Several striking, if less dramatic, studies in a number of organisms have shown that in animals[1], including *Drosophila*[2,3] mice[4,5] and cows[6], size regulation depends predominantly on the proper control of cell growth and division. Now, work has provided evidence that in the eukaryotic social amoeba *Dictyostelium discoideum*, one of the simplest organisms used to study multicellular development, a cell-counting mechanism is used to regulate size. Although multicellularity in *Dictyostelium* results from the aggregation of a large number of individual cells, as described below, rather than cell growth and division as in metazoans, size regulation mechanisms in all multicellular organisms must address similar conceptual problems: tissue size must be measured, compared with a critical value, and adjusted appropriately.

Given sufficient nutrients, *Dictyostelium* is unicellular and divides by binary fission. However, depletion of the food supply triggers a survival mechanism. The formation of a multicellular organism is mediated by the chemotactic aggregation of up to ~$10^5$ cells to form a hemispherical mound[7,8]. Subsequent morphogenesis and cell-type differentiation leads to the production of a fruiting body that consists of a ball of dormant spores, held aloft by a rigid stalk constructed of dead, vacuolated cells (Fig. 1). The purpose of *Dictyostelium* multicellular development, survival under famine conditions, is best served by optimizing the production and dispersal of spores. Therefore, a balance must be struck between differentiating as many spores as possible and forming a stalk that is sufficiently tall and strong to elevate the spore mass above the substrate and assist the dispersion of spores to an environment that is richer in food. Work in many laboratories has shown that this is partially accomplished by a homeostasis mechanism that maintains a constant ratio of presumptive spore cells to presumptive stalk cells[7,9,10]. Removal of a portion of a *Dictyostelium* organism at any time before terminal differentiation induces redifferentiation of the remaining cells to correct the aberrant cell-type ratio, ensuring a correctly proportioned fruiting body, irrespective of organism size. However, the observation that, given a sufficient density of cells, wild-type aggregates tend to be fairly uniform, suggests that a mechanism exists to restrict aggregate size to within an optimal range. If an aggregate is constructed of too-few cells, the resulting fruiting body might be compromised in its efficiency to deliver spores to a new source of nutrients. A fruiting body that is too large, on the other hand, might have a stalk that collapses under the weight of the spore mass. In addition, if the spore mass is too large, its weight might cause it to slide down the stalk (Fig. 2). Finally, evidence suggests that morphogenesis and spatial patterning are regulated by oscillatory cAMP waves that are initiated at the apical tip of the mound and relayed outward[11]. Experiments in which these cAMP oscillations are reduced by chemical inhibition[12] suggest that, if the number of cells in the aggregate is too high, this organizational mechanism might not function effectively to ensure proper fruiting-body formation.

**FIGURE 1. *Dictyostelium discoideum***




A mature fruiting body, 24 hours after the initiation of multicellular development. Scale bar represents 2.1mm.

A recently published study helps to explain how the size of *Dictyostelium* multicellular organisms is regulated. A screen for mutants that form abnormally small aggregates identified the gene *smlA* (Refs 13, 14). Cells containing a targeted deletion of *smlA* initially form normal-sized aggregation domains but then break up into small groups of only ~$5 \times 10^3$ cells, a fraction of the $10^5$ cells found in wild-type aggregates developed under similar conditions. *smlA* encodes a novel 35-kDa protein that is found in the cytosol of all cells during vegetative growth and early development. To examine

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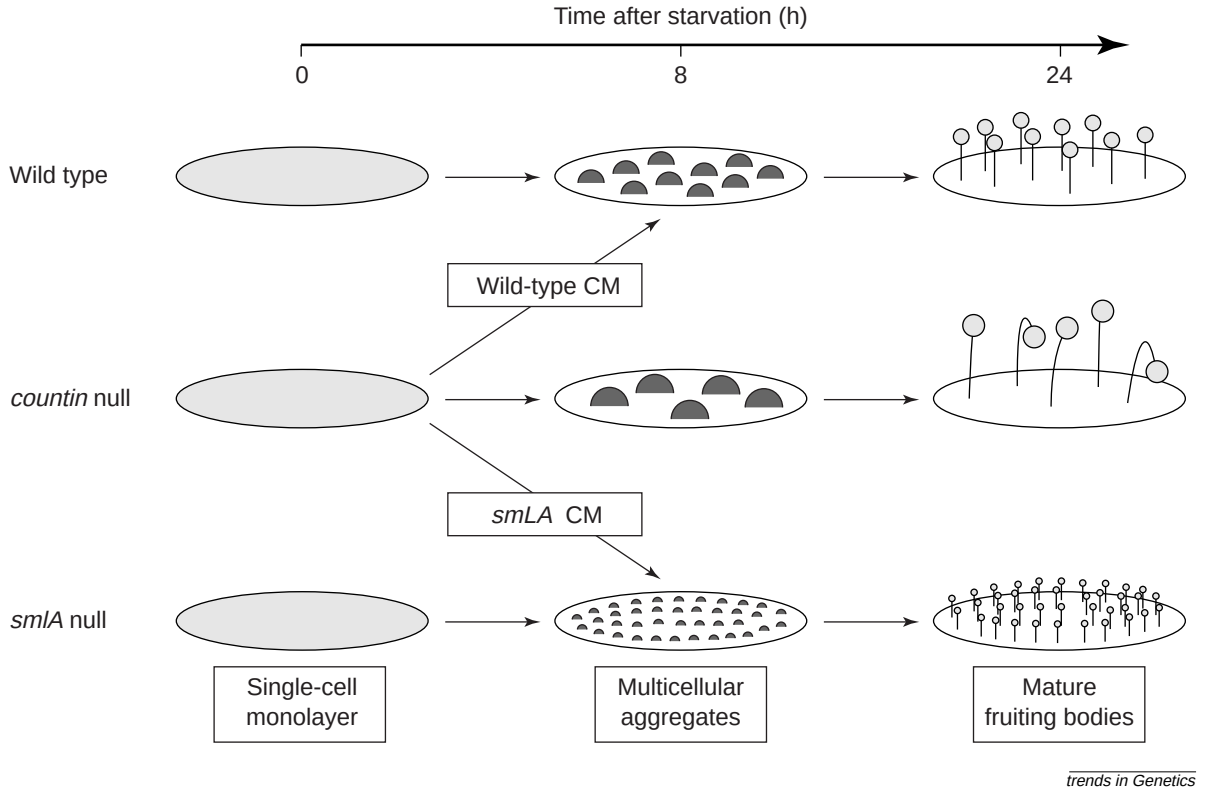
**FIGURE 2. Multicellular development of wild-type and mutant *Dictyostelium* strains**




*smlA* null cells hypersecrete a large, macromolecular 'counting factor' that causes the formation of smaller than normal aggregates. *countin* null cells contain a disruption of a counting factor subunit that results in the formation of abnormally large aggregates. Secretion of counting factor is assayed using conditioned medium (CM) experiments. CM is made by starving cells in non-nutrient buffer to induce aggregation-stage responses. After several hours, the cells are removed by centrifugation, leaving behind any factors secreted during starvation. Development of *countin* null cells in the presence of CM in which wild-type cells have been starved causes a decrease in aggregate size. *countin* null cells developed in the presence of *smlA* null cell CM produce even smaller aggregates, probably because of the hypersecretion of counting factor by *smlA* null cells compared with wild-type cells. Note that the sizes of the aggregates are drawn roughly to scale; the size of aggregates induced by CM might not be exactly as observed for wild-type or *smlA* null cells.

the possibility that an *smlA* loss-of-function mutation affects an extracellular factor that governs aggregate size, *smlA* null cells were mixed with wild-type cells and allowed to co-aggregate and develop as a chimeric organism. Surprisingly, the presence of only 5% *smlA* null cells in a chimera is sufficient to cause a predominantly wild-type group of cells to form small aggregates. Further studies demonstrated that exposure of wild-type cells to conditioned medium (CM), in which *smlA* null cells had previously been starved to induce aggregation stage responses *in vitro* (see Fig. 2), also resulted in the formation of abnormally small aggregates. These results support the model that the secretion of some factor or factors involved in the regulation of aggregate size is altered in *smlA* null cells.

When Brock and Gomer[15] purified the activity secreted by *smlA* null cells that is responsible for reducing aggregate size, they found it to be a large complex (>400 kDa) that contains at least six polypeptides[16]. The authors also found that the complex is secreted by wild-type cells, as expected, but at a much lower level. These observations suggested that this complex acts as a 'counting factor,' the concentration of which is used by developing cells to monitor aggregate size. If this were the case, hypersecretion of the counting factor by *smlA* null cells would be expected to cause aggregates to break up into smaller domains, owing to the perception by the aggregates that their sizes are too large. The results of Brock *et al.*[14,15] are consistent with this model. It is possible, but not known, that the specific activity of the complex could also be increased in *smlA* null cells.

A prediction of this model is that reducing the concentration of counting factor should have the opposite effect, causing abnormally large aggregates to form. The cloning of the *countin* gene, which encodes one of the protein subunits of the counting factor, enabled the authors to test this hypothesis. When strains containing a targeted disruption of *countin* are allowed to develop, abnormally large aggregates form, as postulated by the model. Such aggregates form fruiting bodies with very long stalks, which often collapse under the weight of the enlarged spore mass. Conditioned medium experiments indicate that deletion of the *countin* gene does not affect the ability of cells to respond to exogenously added counting factor. Treatment of *countin* null cells with wild-type cell CM (which contains counting factor) causes the *countin* null cells to form an increased number of smaller, more normally sized aggregates. Addition of CM from *smlA* null cells induces the formation of even more (and smaller) aggregates, demonstrating that the response is not only functional but also concentration dependent. As expected, development of *countin* null cells in the presence of

*countin* null cell CM has no effect on the phenotype. Finally, pre-adsorption of *smlA* null cell CM with antibodies against countin reduces the activity of the counting factor by half, providing further evidence that aggregate size is indirectly proportional to counting factor concentration. Figure 2 shows the phenotypes of the mutants and the effects of treatment with conditioned medium.

Although *countin* is only one of several polypeptides that make up the counting factor, it clearly plays an important, but undefined, role in the function of the complex. The absence of *countin* might cause a severe reduction in the amount of counting factor secreted. Alternatively, the lack of *countin* might cause a decrease in the specific activity of the complex, rather than affecting the rate of secretion. After this has been resolved, it will be interesting to see if either a complete block of counting factor secretion or disruption of additional subunits of the complex can cause the formation of even larger aggregates. Unfortunately, the sequence of the *countin* protein does not provide any clues as to whether it might be important for receptor interaction, or perhaps function as a scaffolding protein required for the proper conformation of the counting factor.

Several other *Dictyostelium* strains form abnormally small aggregates. For instance, hyperactivation of extracellular phosphodiesterase disrupts the cAMP signaling that is required for chemotaxis, leading to small aggregation domains[16,17], but this does not appear to be the cause of the *smlA* null phenotype[14]. Worthy of closer examination is the observation that mutation of various cell-surface (over expression of gp80)[18] or signaling proteins (disruption of the gene that encodes the MAP kinase kinase MEK1)[19] also results in a similar size reduction. Use of CM from mutant strains combined with genetic studies will help to determine if the phenotype of any of these strains is owing to a functional relationship with the counting factor.

The identification of a dose-dependent, size-modulation system in *Dictyostelium* is an exciting step toward understanding the mechanisms that regulate the size of multicellular organisms. This work leaves us with many questions. What other proteins make up the counting factor complex and how does *smlA* affect its secretion? What are the receptor(s) and signaling pathways that allow cells to detect and react to counting factor in a concentration-dependent manner? How does the signal that an aggregate is too large lead to physical separation into smaller domains? Continued study of the composition and regulation of counting factor, as well as the examination of mutants with similar phenotypes, will help to determine how aggregate size in *Dictyostelium* is modulated and might yield further insight into the regulation of tissue size in other multicellular organisms.

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# Dyskeratosis congenita, telomeres and human ageing

**As normal humans age, telomeres shorten in tissues that contain dividing cells, and this has been proposed both as a cause of ageing and as a tumor-suppressor mechanism. The surprising finding that cells from individuals with the rare inherited disorder dyskeratosis congenita (DKC) have reduced levels of telomerase and shortened telomeres might provide the first direct genetic test of the function of telomeres in intact humans.**

DKC is a human genetic disorder with variable modes of inheritance, including a predominant X-linked form (reviewed in Refs 1, 2). Initial clinical manifestations are abnormalities in skin pigmentation and nail growth that become apparent by the age of ten. Symptoms found in a fraction of individuals with DKC include blocked tear ducts, learning difficulties, pulmonary disease, graying and loss of hair, abnormalities of the gastrointestinal tract, hypogonadism and osteoporosis. An increased incidence of epithelial and hematological cancers is also observed. Death occurs at a median age of 16 years and a maximum of approximately 50 years, most often from bone-marrow