A 'Dynamic Adder Model' For Cell Size Homeostasis in Dictyostelium Cells

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Title

A 'dynamic adder model' for cell size homeostasis in *Dictyostelium* cells

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Abstract

After a cell divides into two daughter cells, the total cell surface area of the daughter cells should increase to the original size to maintain cell size homeostasis in a single cell cycle. Previously, three models have been proposed to explain the regulation of cell size homeostasis: sizer, timer, and adder models. Here, we precisely measured the total cell surface area of *Dictyostelium* cells in a whole cell cycle by using the agar-overlay method, which eliminated the influence of surface membrane reservoirs, such as microvilli and membrane winkles. The total cell surface area linearly increased during interphase, slightly decreased at the metaphase, and then increased by approximately 20% during cytokinesis. From the analysis of the added surface area, we concluded that the cell size was regulated by the near-adder model in interphase and by the timer model in the mitotic phase. The adder model in the interphase is not caused by a simple cell membrane addition, but is more dynamic due to the rapid cell membrane turnover. We propose a ‘dynamic adder model’ to explain cell size homeostasis in the interphase.

Introduction

What determines the size of cells is a large question for cell biologists [1,2,3]. Generally, the cellular size is the smallest immediately after cell division (at birth); the daughter cells grow during the cell cycle and become the largest just before the next division, in which cells maintain their original cell size. Cell size homeostasis has been mainly studied in yeasts and bacteria, because their cell shapes are relatively simple, and their size can be easily measured [4,5,6]. Studies of these cells have shown that they regulate the growth rate during the cell cycle in such a way that larger cells at birth divide earlier than smaller ones, and vice versa [7,8].

There are three models to explain the regulation of cell size homeostasis: sizer, timer, and adder models. The sizer model has been reported for fission yeast and budding yeast daughter cells, whose cells divide after growing to a certain size [9,10]. The timer model has been reported for *Caulobacter crescentus*, whose cells divide after a certain time [11]. Adder models have been reported for *Escherichia coli, Bacillus subtilis*, and budding yeast mother cells, whose cells divide after a certain amount of cell size (cell volume, cell surface area, among others) is added, independently of their initial size [4,10,12]. As bacteria and yeasts have cell walls, the regulation of their cell size includes the regulation of cell walls. Although measuring the size of mammalian cells is difficult because they have an irregular shape, recent studies have measured the cell volume by using fluorescence exclusion and microfabricated channels and showed that their cell size is regulated by adder model or near-adder model [13,14]. However, because the cell volume tends to change depending on the intracellular hydrostatic pressure, the total cell surface area could be a more reliable parameter of cell size [13,14,15,16].

The precise measurement of the total cell surface area is more difficult because of small microvilli or wrinkles on the cell surface in animal cells, which occupy 21% – 130% of the apparent cell surface area [17,18]. Recently, we developed a method to precisely measure the total cell surface area, via which cells are flattened by overlaying with an agar block, which expands the small protrusions and wrinkles present on the cell surface [19].

Here, by using the agar-overlay method, we examined the dynamics of the total cell surface area in the whole cell cycle of *Dictyostelium* cells, a model organism for cell growth, cell division, and cell migration. From the analysis of the added surface

area, the cell surface area at birth, and the generation time, we found that the cell size homeostasis in the interphase and mitotic phase of *Dictyostelium* cells is regulated by the near-adder and timer models, respectively. The adder model in the interphase was not as simple as previously discussed, being considerably dynamic due to the rapid turnover of the cell membrane. We propose a ‘dynamic adder model’ to explain cell size homeostasis.

Results

Long period-observation of cells under an agar-overlay

Previously, we developed a method to precisely measure the total cell surface area, in which cells are flattened by overlaying with an agar block, which expands the small protrusions and wrinkles of the cell surface [19,20]. To examine the dynamics of the total cell surface area in a whole cell cycle, cells were observed under an agar-overlay for a long period (up to 12 h). In our early experiments, we used an agar block containing a nutrient medium (HL5), but the cell size was gradually reduced, presumably because cells did not fully uptake HL5 medium from the agar block [21]. Since *Dictyostelium* cells grow by phagocytosing bacteria in a natural habitat, cells were observed in the presence of live bacteria (*Escherichia coli*) under an agar-overlay (Fig. 1A). In these conditions, the doubling time was approximately 4 h (3.87 ± 1.15 h, n = 147), which is consistent with the previous observation in a suspension culture in the presence of bacteria [22]. It has been reported that in a confined space, such as a microchannel, mammalian cells tend to asymmetrically divide, creating siblings with different sizes [13,14]. However, *Dictyostelium* cells are normally and symmetrically divided under an agar-overlay. Figure 1B shows the differences in the total cell surface area between the siblings (7.02% ± 5.94%, n = 227). Therefore, we established a method to observe cells under an agar-overlay for a long period.

Dynamics of total cell surface area in whole cell cycle

Figure 1C shows the typical time course of a single cell surface area in a whole cell cycle. The total cell surface area linearly increases after cytokinesis (red mark on the left), subtly decreases at mitosis (green mark on the right), increases by approximately 20% during cytokinesis, and decreases after cell division (blue mark on the right). Figure 1D shows a graph of the relative cell surface area versus the relative cell cycle time in multiple cells (n = 147). The relative cell cycle time was normalized from the first division to the second division time. *Dictyostelium* cells have a prolonged G2 phase that accounts for over 90% of the cell cycle, and M and S phases account for approximately 10% [23]. Thus, from birth before the next mitosis, the cells are almost in G2 phase. The cell surface area linearly increased during G2 phase; thereafter, it subtly decreased at mitosis (6.0% ± 2.11%) and increased by about 20% (21.64% ± 3.11%)

during cytokinesis (n = 147). The changes in the cell surface area during mitosis and cytokinesis were consistent with our previous observations [19].

When the cells were divided into three populations: larger (> 450 μm^2, green), average (400 ± 50 μm^2, black), and smaller size (< 350 μm^2, red) (Fig. 1E), the larger cells significantly decreased in size after the second cell division compared with after the 1st cell division. On the other hand, smaller cells significantly increased in size after the second cell division compared with after the first cell division. Figure 1F shows the time courses of individual cells, suggesting that larger cells (green) divided much faster than smaller cells (red). These results suggest that *Dictyostelium* cells have a cell size homeostasis mechanism that makes cells of a deviated size return to the average size.

Cell surface area in *Dictyostelium* cells is regulated by near-adder model

There are three models to explain the regulation of cell size homeostasis: sizer, timer, and adder models. These models can be assessed by quantifying the total cell surface areas at birth and immediately before the 2nd cell division, the added surface area between them, and the generation time [7,8,24].

Figures 2A-C show ideal graphs to assess the three models. Figure 2A shows graphs of the added surface area during a single cell cycle versus the cell surface area at birth. If the slope is +1, the model should be the timer model (green). If the slop is 0, the model should be the adder model (red). If the slope is −1, the model should be the sizer model (blue). Figure 2B shows graphs of the cell surface area immediately before cytokinesis versus the cell surface area at birth. If the slope is +1, the model should be the adder model. If the slope is 0, the model should be the sizer model. Figure 2C shows graphs of the logarithmic generation time versus the logarithmic cell surface area at birth. If the slope is -1, the model should be the adder model (red). If the slope is 0, the model should be the timer model (green). These graphs were plotted as described in the Methods section.

Figure 2D shows actual plots for *Dictyostelium* cells with respect to the added surface area during the whole cell cycle versus the cell surface area at birth, indicating that the slope was -0.15. Figure 2E shows actual plots of the cell surface area immediately before cytokinesis versus the cell surface area at birth, indicating that the slope was 0.85. Figure 2F shows actual plots of the logarithmic generation time versus

the logarithmic cell surface area at birth, indicating that the slope was -1.24. These results suggest that the cell size in *Dictyostelium* is regulated by an adder or near-adder model.

Cell size during the mitotic phase is regulated by timer model

Here, these model assessments were performed during the whole cell cycle, including the interphase and the mitotic phase. Previous model assessments in other cells have been conducted during the interphase but not during the mitotic phase. Figures 2G-I show that similar assessments were conducted only for the interphase. The slopes of the individual assessments were almost similar to those of the whole cell cycle, suggesting that the cell size is regulated by an adder or near-adder model in the interphase.

Next, we examined the cells in the mitotic phase. Figure 2J shows the logarithmic cell division time in the mitotic phase versus the logarithmic surface area. The cell division time in the mitotic phase was the duration from cell rounding to final abscission. The cells took a similar time to divide independently of the cell size, suggesting that the cell size during the mitotic phase is regulated by the timer model.

The cell surface area is maintained by a dynamic balance between exocytosis and endocytosis

The increase in the cell surface area is not caused by the simple addition of the membrane via exocytosis. In the present conditions, cells constantly endocytosed bacteria, suggesting that cells internalized massive membranes. Figure 3A shows the phase-contrast and fluorescence images of cells expressing ABD (actin binding domain of *Dictyostelium* filamin), an F-actin marker, which were observed in the presence of bacteria. Actin filaments were localized at the phagocytic cups (arrows), suggesting that the cells vigorously engulfed the bacteria. The number of internalized bacteria was examined by staining with DAPI (4',6-diamidine-2'-phenylindole dihydrochloride) after fixation (Fig. 3B and C). Approximately 19 (19.1 ± 4.1, n = 87) bacteria were found in individual cells, which is consistent with previous observations [25,26]. Incidentally, only approximately 1.5 (1.53 ± 1.38, n = 88) bacteria were found in mitotic cells, indicating that phagocytosis was significantly reduced in the mitotic phase, which has not been reported in *Dictyostelium* cells.

To examine the amount of internalized cell membrane during phagocytosis, the fluorescence intensities of the cells in the presence of FM1-43, which emits fluorescence when inserted into the lipid bilayer, were measured using fluorescence spectrophotometry. Since the cell membranes of bacteria were also stained with FM dye, the fluorescence intensities of bacteria were subtracted from those of *Dictyostelium* cells with bacteria in their interior. Figure 3D shows that membrane uptake was significantly increased when cells internalized bacteria. Figure 3E shows the time course of the total cell surface area in the presence of bacteria, indicating that it was almost constant, in spite of the vigorous internalization of cell membranes; therefore, exocytosis should compensate for the cell membrane that was lost during phagocytosis.

Together, the total cell surface area is maintained by a dynamic balance between exocytosis and endocytosis.

Turnover of cell membrane may regulate cell size homeostasis

Dictyostelium cells grow faster in the presence of bacteria than in axenic HL5 medium [22]. We expected that the increased cell membrane turnover induced cells to grow faster in the presence of bacteria. To examine whether the total cell surface area increased faster in the presence of bacteria than in HL5 medium, partially synchronized cells were cultured in HL5 medium or in the presence of bacteria. To synchronize the cells, they were cultured at 10°C for 16 h, and then arrested at the metaphase via treatment with 100 μM thiabendazole (TB), a microtubule depolymerizer, at 22°C for 3.5 h [27]. After the mitotic arrest was released by removing the TB, the time courses of the total cell surface area were examined using the agar-overlay method, followed by fixation. In the presence of bacteria, the cell surface area peaked at 4 h (green asterisk), which corresponds to the generation time in the presence of bacteria. In HL5 medium, it peaked at 8 h (red asterisk), which corresponds to the generation time in the presence of HL5 medium. Therefore, it is plausible that the increased turnover of cell membrane enhances the cell growth rate; therefore, cell size homeostasis may be regulated by the cell membrane turnover rate.

Discussion

Here, we precisely measured the total cell surface area in a whole cell cycle by using the agar-overlay method. We found that the total cell surface area increased linearly during the interphase without any specific time point of increase. The cell size homeostasis in the interphase and mitotic phase of *Dictyostelium* cells is regulated by the adder model and timer model, respectively. During the interphase, cells regulate the growth rate; larger cells divide earlier than average-sized cells and smaller cells divide later (Fig. 1F).

In the adder model, cells divide after a certain amount of cell membrane or cell volume is added [4,10,12]. The previous adder model did not take into account the cell membrane turnover but only its addition (Fig. 4A). However, the cell membrane rapidly turns over in migrating cells [20]. In addition, if cells phagocytose 20 bacteria per 10 min, the internalized cell membrane area is about 180 μm^2, which is equivalent to 30% – 60% of the total cell membrane. If the duration of the interphase is 220 min, the whole cell membrane should be refreshed 7 – 13 times. However, the total cell surface area only doubled, suggesting that exocytosis compensates for the amount of membrane lost via endocytosis. Therefore, in the adder model, the added surface area should be determined by subtracting the amount of membrane uptake via endocytosis from that of membrane supply via exocytosis (Fig. 4B).

Cells must always monitor their cell size and regulate it via endocytosis and exocytosis. As a simple explanation for this mechanism, they may monitor the added amount of cell membrane components. As reported in animal cells, cells begin to divide by sensing the ratio of membrane components, such as cholesterol, in the cell membrane [28].

Furthermore, the cell membrane turnover rate may regulate the cell size (Fig. 4C). When cells were cultured in the presence of bacteria, the rate of cell membrane turnover increased, and the generation time decreased (Fig. 3F).

Additionally, cortical tension may contribute to monitoring. Previous studies have reported that exocytosis and endocytosis are regulated by cortical tension [29,30,31]. Cortical tension may increase as the cell size increases, which may trigger the preparation for cell division.

We found that the timer model is suitable for the cell size regulation in the mitotic phase in *Dictyostelium* cells. Generally, animal eggs divide repeatedly without G1 and G2 phases in early development, and the cell size becomes half of the original each time the cell divides. Therefore, the eggs can be divided without a large increase in cell size. As the rate of DNA synthesis (S phase) is fairly constant, these cells may regulate the cell size in the mitotic phase. Their cell size may also be regulated by a timer model in the mitotic phase, as observed for *Dictyostelium* cells in the present study.

Figure 4D summarizes the dynamics of the total cell surface area in the cell cycle of *Dictyostelium* cells. We propose a ‘dynamic adder model’ to explain cell size homeostasis in the interphase. To explain the constant increase in cell surface area during the interphase, exocytosis should slightly exceed endocytosis in the dynamic turnover of the cell membrane. The molecular mechanism underlying the precise regulation of cell size remains to be clarified.

Methods

Cell Culture

Dictyostelium discoideum wild type (AX2) cells were cultured in a plastic dish at 22°C in HL5 medium (1.3% bacteriological peptone, 0.75% yeast extract, 85.5 mM D-glucose, 3.5 mM Na_2HPO_4 $12H_2O$, 3.5 mM KH_2PO_4, pH 6.3). An extra-chromosomal expression vector of GFP-ABD (actin binding protein of *Dictyostelium* filamin) was transformed into cells via electroporation or laserporation, as described previously [32,33]. Transformed cells were selected in HL5 medium supplemented with 10 μg/mL G418 (Wako, Osaka, Japan). For the shaking culture, the cells were cultured in conical flasks (100 mL) containing 20 mL of HL5 medium or 20 mL of a Na/K-phosphate buffer containing *Escherichia coli* (*E. coli, B/r*) at 22°C in a reciprocal shaker at 150 rpm. To synchronize the cell cycle to increase the number of mitotic cells, cells were cultured at 10°C for 16 h and treated with 100 μM thiabendazole (TB) at 22°C for 3.5 h [27]. *E. coli* was cultured in HL5 medium in suspension at 37°C and washed with a 15 mM Na–K phosphate buffer (pH 6.3) using centrifugation.

Microscopy

Dictyostelium cells and bacteria were placed in a glass-bottom dish and overlaid with an agarose block [34]. After the agar-overlay, the cells were observed under an optical sectioning fluorescence microscope (Deltavision, GE Healthcare Life Science, United Kingdom). The images were acquired over time and stitched from 12 images stacks arranged in a 4 × 3 grid (total area is 824 × 612 μm.) every 1 min.

To observe the fixed cells, the agar-overlaid cells were fixed by immersing in ethanol containing 1% formaldehyde at -17°C, as described previously [34]. Fixed cells were stained with DAPI (Sigma-Aldrich, Tokyo, Japan) and tetramethyl rhodamine (TRITC)-phalloidin (Sigma-Aldrich) and observed under a fluorescence microscope (TE 300, Nikon, Japan) equipped with regular UV and TRITC filter sets.

Fluorescence images of live cells expressing GFP-ABD in the presence of *E. coli* were acquired using a confocal microscope (LSM510, Zeiss, Germany) at time intervals of 20 s. The total cell surface areas were calculated from the cell outline and thickness by using ImageJ, as described previously [19]. All images were processed and analyzed using ImageJ software.

Assessment of three models for cell size homeostasis

Three models for cell size homeostasis (sizer, timer, and adder models) were assessed based on the slopes in Figure 2A-C and on previous reports [7,8,24]. When cell size homeostasis is regulated by perfect sizer, timer, or adder models, the relationship between the added surface area during a single cell cycle (y) versus the cell surface area at birth (x) for the three models is the following:

adder model: $y = C_{AVE}$ (1)

sizer model: $y = 2C_{AVE} - x$ (2)

timer model: $y = x$ (3)

Where C_{AVE} is the average size at birth. As shown in Figure 2A, the slope is 0 for the adder model, -1 for the sizer model, and +1 for the timer model.

The relationship between the cell surface area immediately before cytokinesis (y) and the cell surface area at birth (x) for the sizer and adder models is the following:

adder model: $y = x + C_{AVE}$ (4)

sizer model: $y = 2C_{AVE}$ (5)

As shown in Figure 2B, the slope is +1 for the adder model and 0 for the sizer model.

The relationship between the generation time and the cell surface area at birth for the timer and adder models is the following:

timer model: generation time = T_{AVE} (6)

adder model: generation time = C_{AVE} T_{AVE}/cell surface area at birth (7)

Where T_{AVE} is an average generation time.

To obtain the slopes of linear lines, both sides of the equations were converted to logarithms (y: logarithmic generation time, x: logarithmic cell surface area at birth), as follows:

timer model: $y = \log T_{AVE}$ (8)

adder model: $y = \log C_{AVE} + \log T_{AVE} - x$ (9)

As shown in Figure 2C, the slope is 0 for the timer model and -1 for the adder model.

Uptake of Cell membranes

The uptake of cell membranes was measured by staining cells with 10 μM FM1-43 (Thermo Fisher Scientific, Tokyo, Japan), a fluorescent lipid analog. Since the nutrient

medium hampered the staining, the cells were stained after the medium had been exchanged with 15 mM Na/K phosphate buffer (pH 6.4) containing 0.1 M sorbitol. Sorbitol was used to suppress the activity of contractile vacuoles [35]. Ten minutes after staining, cells were washed twice with a Na/K phosphate buffer containing 15 mM sodium azide to suppress exocytosis. The fluorescence intensities (excitation at 470 nm and emission at 570 nm) were measured using a fluorescence spectrophotometer (F-2500, Hitachi High-Technologies, Corp., Tokyo, Japan).

To quantify the uptake of the cell membrane during phagocytosis, *Dictyostelium* cells (4×10^6 cells/mL) were mixed with *E. coli* cells (1×10^8 cells/mL) in Na/K phosphate buffer. Since the cell membrane of *E. coli* was also stained, the fluorescence intensity of *E. coli* cells was subtracted from that of *Dictyostelium* cells containing bacteria.

Statistical analysis

Statistical analysis and linear regression analysis were conducted using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, United States). Data are presented as mean ± SD and analyzed using unpaired two-tailed Student's t-test or one-way ANOVA with Tukey's multiple comparison test.

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Competing interests

The authors declare no competing interests.

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Author contributions

MT was involved in the experimental work and data analysis. SY was involved in the project planning and data analysis. MT and SY wrote the manuscript.

Data availability

All relevant data are available from the authors on reasonable request.

Figure legends

Figure 1. Control of the total cell surface area in a whole cell cycle.

(A) Time course of phase-contrast images of *Dictyostelium* cells in whole cell cycle. The green lines show the mitotic phase, the red lines show cytokinesis, and the blue lines show the cells immediately after cell division (at birth). The cell was outlined with white lines. Scale bar: 10 μm. (B) Differences in surface area between sibling cells. The cells divide almost evenly with a small deviation (7.02% ± 5.94%, n = 227). Data are presented as the mean ± SD. (C) Representative time course of the total cell surface area in whole cell cycle. The colored marks indicate the same events shown in Fig. 1A. (D) Time courses of the total cell surface area from the first birth (blue asterisk) to the second birth (blue points) in multiple cells (n = 147). The relative cell cycle time is normalized. (E) Comparison of the relative cell surface area between the 1st and 2nd cytokinesis and at the 1st and 2nd birth in larger (> 450 μm^2, green), average (400 ± 50 μm^2, black), and smaller sized cells (< 350 μm^2, red). Data are presented as the mean ± SD. **P < 0.001; ns, not significant; P > 0.05. (F) Time courses of the relative cell surface area of individual cells from the first division (red asterisk) to the second division (n = 147). The blue dotted lines show 0.5 and 1.0 of the relative cell surface area, respectively.

Figure 2. Cell surface area in *Dictyostelium* cells is regulated by near-adder model.

(A-C) Ideal graphs to assess the three models to explain the regulation of cell size homeostasis. (A) The added surface area during cell cycle versus the cell surface area at birth. (B) The cell surface area immediately after cytokinesis versus the cell surface area at birth. (C) The logarithmic generation time versus the logarithmic cell surface area at birth. C_{ave} and T_{ave} show the average cell surface area at birth and the average generation time, respectively. (D-F) Graphs of actual data in whole cell cycle (Whole). (G-I) Graphs of actual data between the first cell division and immediately before mitosis (Interphase). (J) A graph of actual data during cell division (M phase). Linear regression lines are shown in red.

Figure 3. The cell surface area is maintained by a dynamic balance between exocytosis and endocytosis.

(A) Representative time courses of phase-contrast, fluorescence, and merge images of a live cell expressing GFP-ABD in the presence of bacteria. Arrows indicate bacteria being internalized by the cells. Scale bar, 10 μm. (B) Representative phase-contrast and fluorescence images of cells stained with DAPI in the presence and absence of bacteria. Many bacteria were found in interphase cells, but a few bacteria were found in mitotic cells. Scale bar, 10 μm. (C) The number of bacteria in cells during interphase and in mitotic cells. Data are presented as the mean ± SD. **P < 0.001; n ≥ 80 for each. (D) Quantitative analysis of the total cell surface area in the presence and absence of bacteria. Cells were stained with FM1-43 and measured using fluorescence spectrophotometry in each condition. Data are presented as the mean ± SD. **P < 0.001, three different experiments. (E) A time course of the total cell surface area in the presence of bacteria (n = 28). (F) Time courses of the cell surface area in HL5 medium and a phosphate buffer containing *E.coli.* Cells were arrested via cold treatment and thiabendazole (TB) at the mitotic stage (Mitosis). After releasing the mitotic arrest by removing TB, the total cell surface area was examined by using the agar-overlay method, followed by fixation at the indicated times. Data are presented as the mean ± SD and analyzed using Student's t-test. **P < 0.001; ns, not significant; P > 0.05, three different experiments.

Figure 4. Dynamic adder model.

(A) A previous adder model considered only the added surface area without taking into account the turnover of cell membrane. Here, ΔS is the total added surface area, and ΔS_{EXO} is the added surface area due to exocytosis. (B) Since the cell membrane rapidly turns over, the total added surface area (ΔS) should be determined by subtracting the amount of membrane uptake by endocytosis (ΔS_{END}) from that of membrane supply by exocytosis (ΔS_{EXO}). (C) The turnover rate of cell membrane (V) may regulate the cell size. V is calculated by dividing the added surface area (ΔS) by a unit of time (ΔT). (D) A summary of the dynamics of the total cell surface area in the cell cycle of *Dictyostelium* cells.

Figure 1

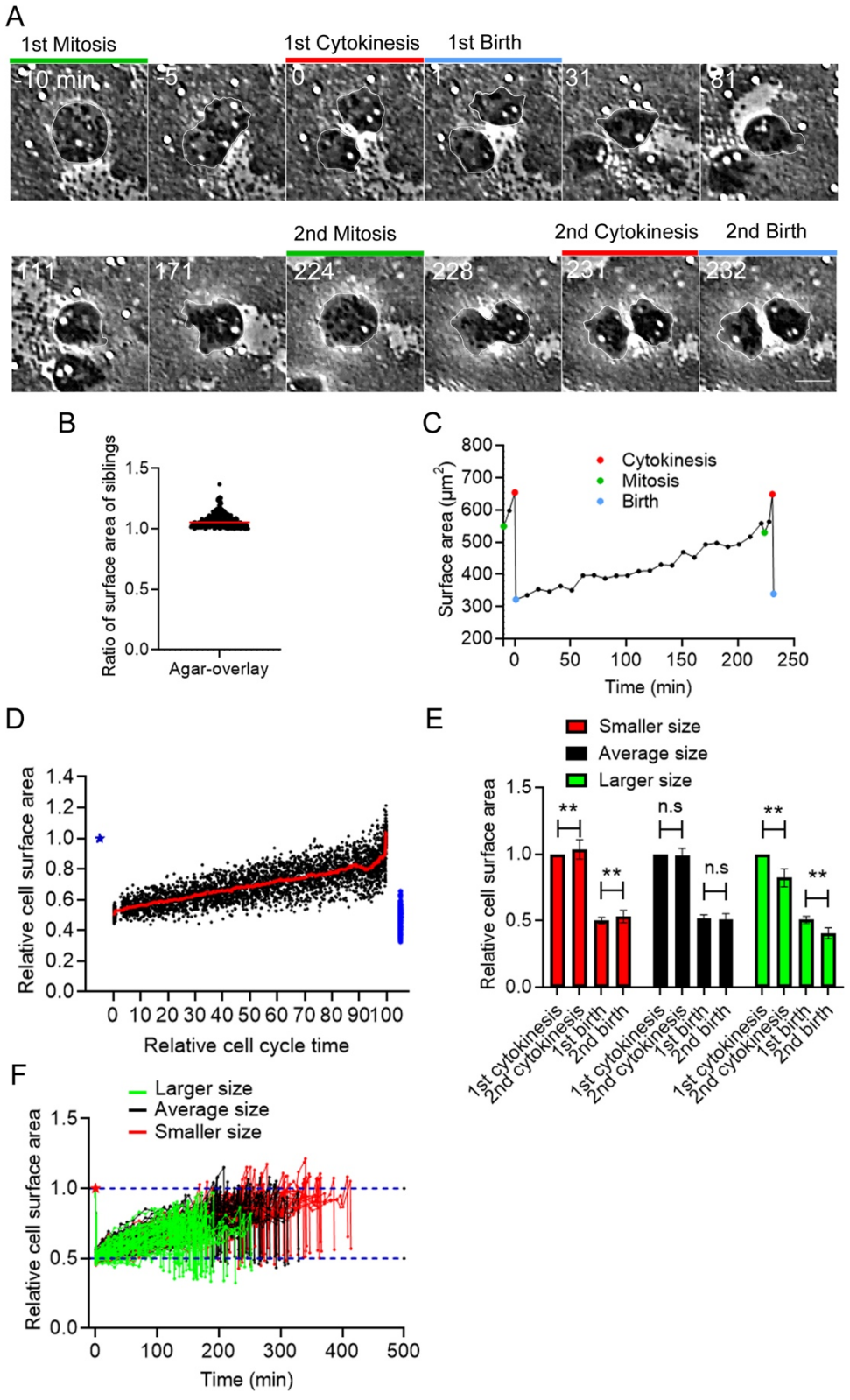

A
1st Mitosis
1st Cytokinesis
1st Birth
-10 min
-5
0
1
31
81
2nd Mitosis
2nd Cytokinesis
2nd Birth
111
171
224
228
231
282
B
Ratio of surface area of siblings
Agar-overlay
C
Surface area (μm²)
Cytokinesis
Mitosis
Birth
Time (min)
D
Relative cell surface area
Relative cell cycle time
E
Smaller size
Average size
Larger size
Relative cell surface area
n.s
1st cytokinesis
2nd cytokinesis
1st birth
2nd birth
F
Larger size
Average size
Smaller size
Relative cell surface area
Time (min)

Figure 2

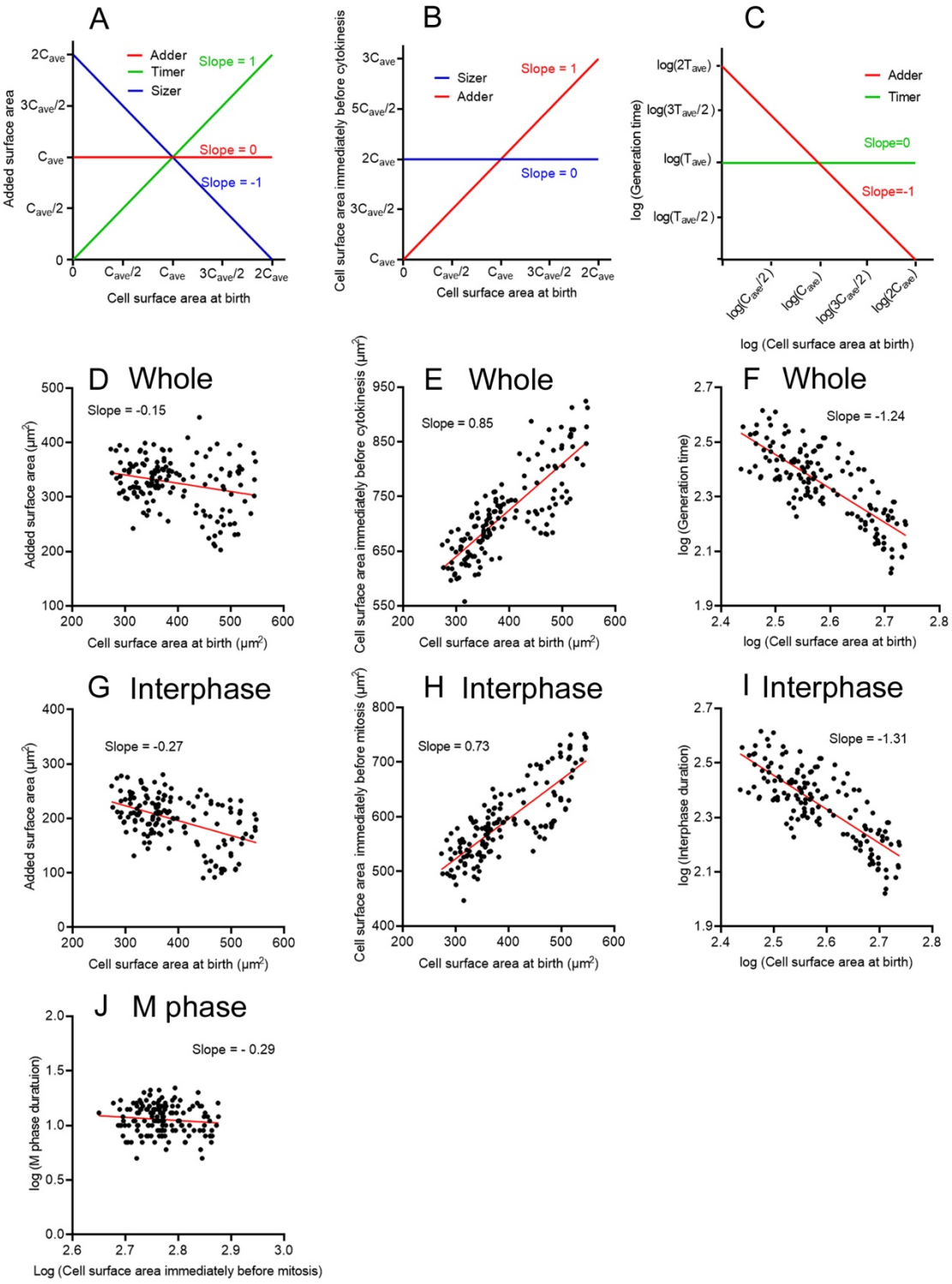

A
Adder
Timer
Sizer
Slope = 1
Slope = 0
Slope = -1
Added surface area
Cell surface area at birth
B
Sizer
Adder
Slope = 1
Slope = 0
Cell surface area immediately before cytokinesis
Cell surface area at birth
C
Adder
Timer
Slope=0
Slope=-1
log (Generation time)
log (Cell surface area at birth)
D Whole
Slope = -0.15
Added surface area (μm²)
Cell surface area at birth (μm²)
E Whole
Slope = 0.85
Cell surface area immediately before cytokinesis (μm²)
Cell surface area at birth (μm²)
F Whole
Slope = -1.24
log (Generation time)
log (Cell surface area at birth)
G Interphase
Slope = -0.27
Added surface area (μm²)
Cell surface area at birth (μm²)
H Interphase
Slope = 0.73
Cell surface area immediately before mitosis (μm²)
Cell surface area at birth (μm²)
I Interphase
Slope = -1.31
log (Interphase duration)
log (Cell surface area at birth)
J M phase
Slope = - 0.29
log (M phase duration)
Log (Cell surface area immediately before mitosis)

Figure 3

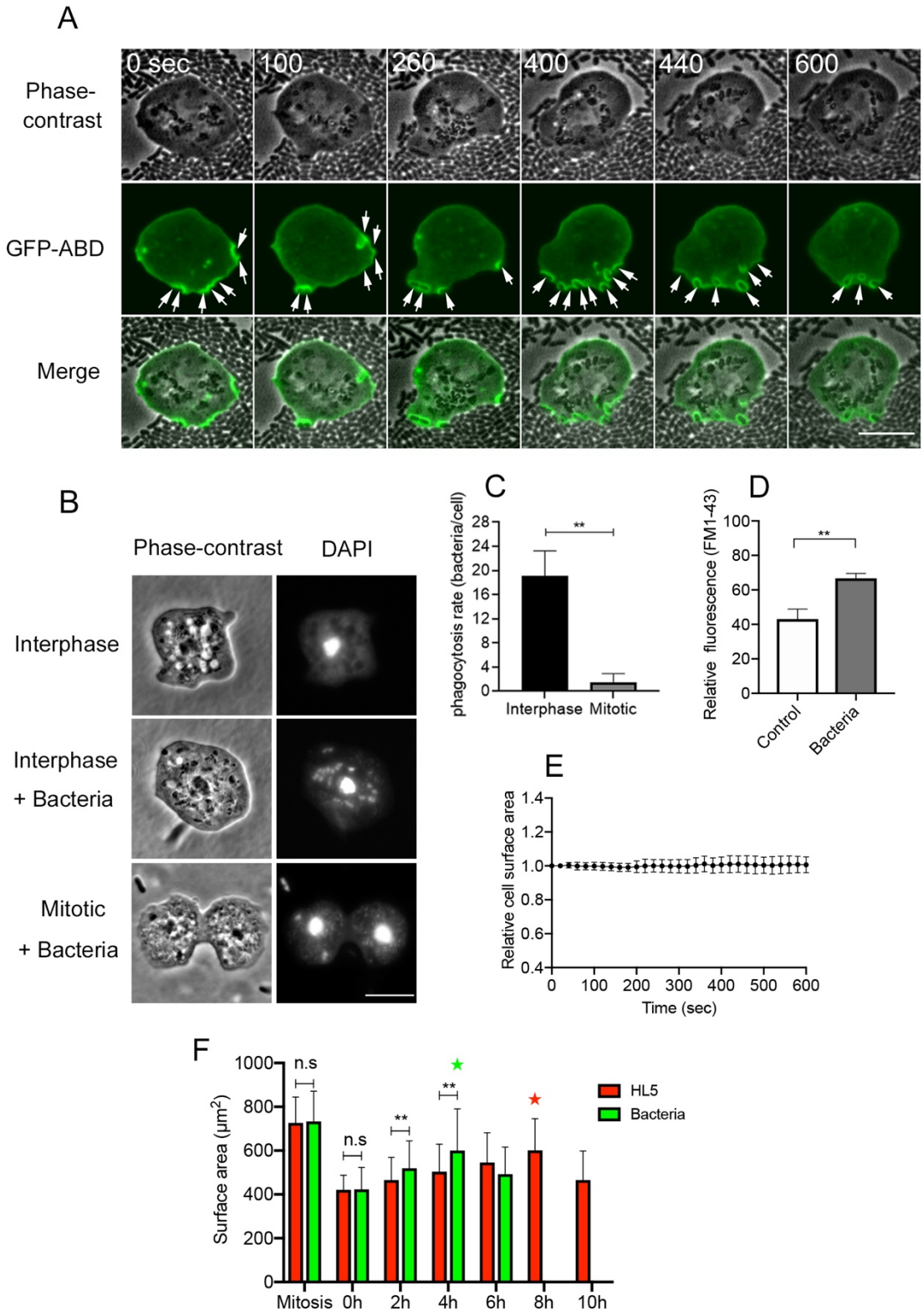
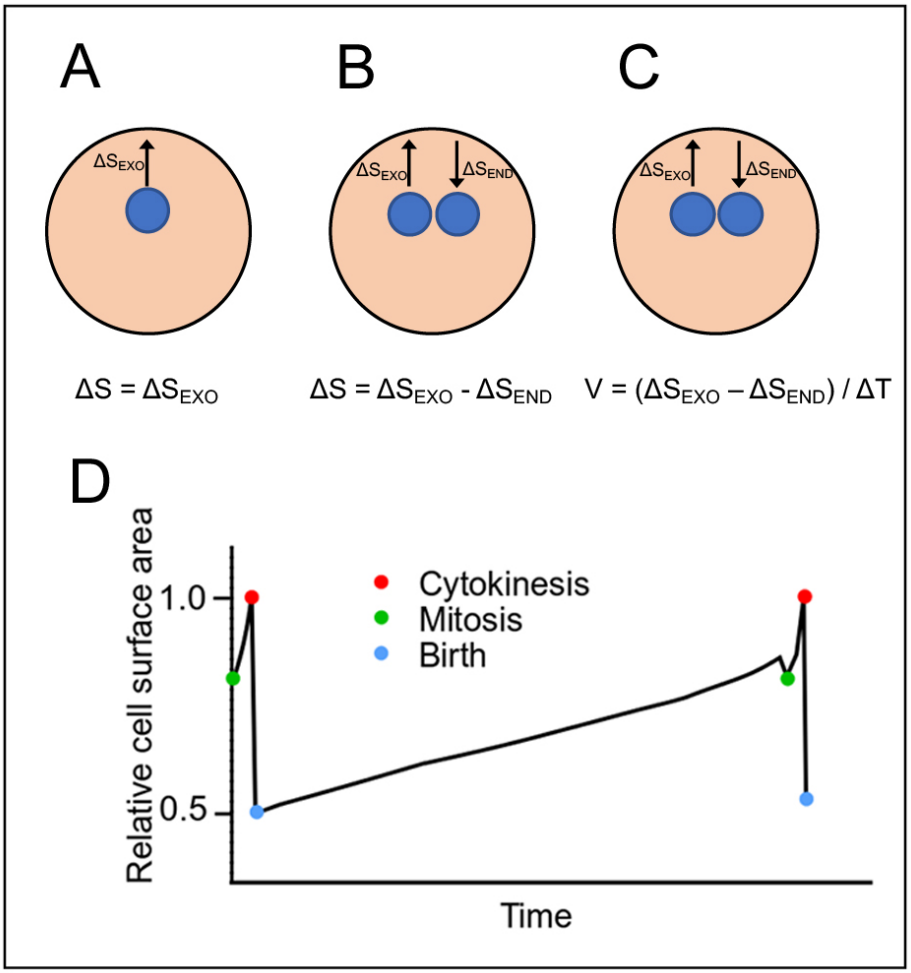

A
0 sec
100
260
400
440
600
Phase-contrast
GFP-ABD
Merge
B
Phase-contrast
DAPI
Interphase
Interphase + Bacteria
Mitotic + Bacteria
C
phagocytosis rate (bacteria/cell)
Interphase
Mitotic
D
Relative fluorescence (FM1-43)
Control
Bacteria
E
Relative cell surface area
Time (sec)
F
Surface area (μm²)
n.s
HL5
Bacteria
Mitosis
0h
2h
4h
6h
8h
10h

Figure 4


A
B
C
ΔS_EXO
ΔS_EXO
ΔS_END
ΔS_EXO
ΔS_END
ΔS = ΔS_EXO
ΔS = ΔS_EXO - ΔS_END
V = (ΔS_EXO – ΔS_END) / ΔT
D
Relative cell surface area
1.0
0.5
Cytokinesis
Mitosis
Birth
Time

Figures

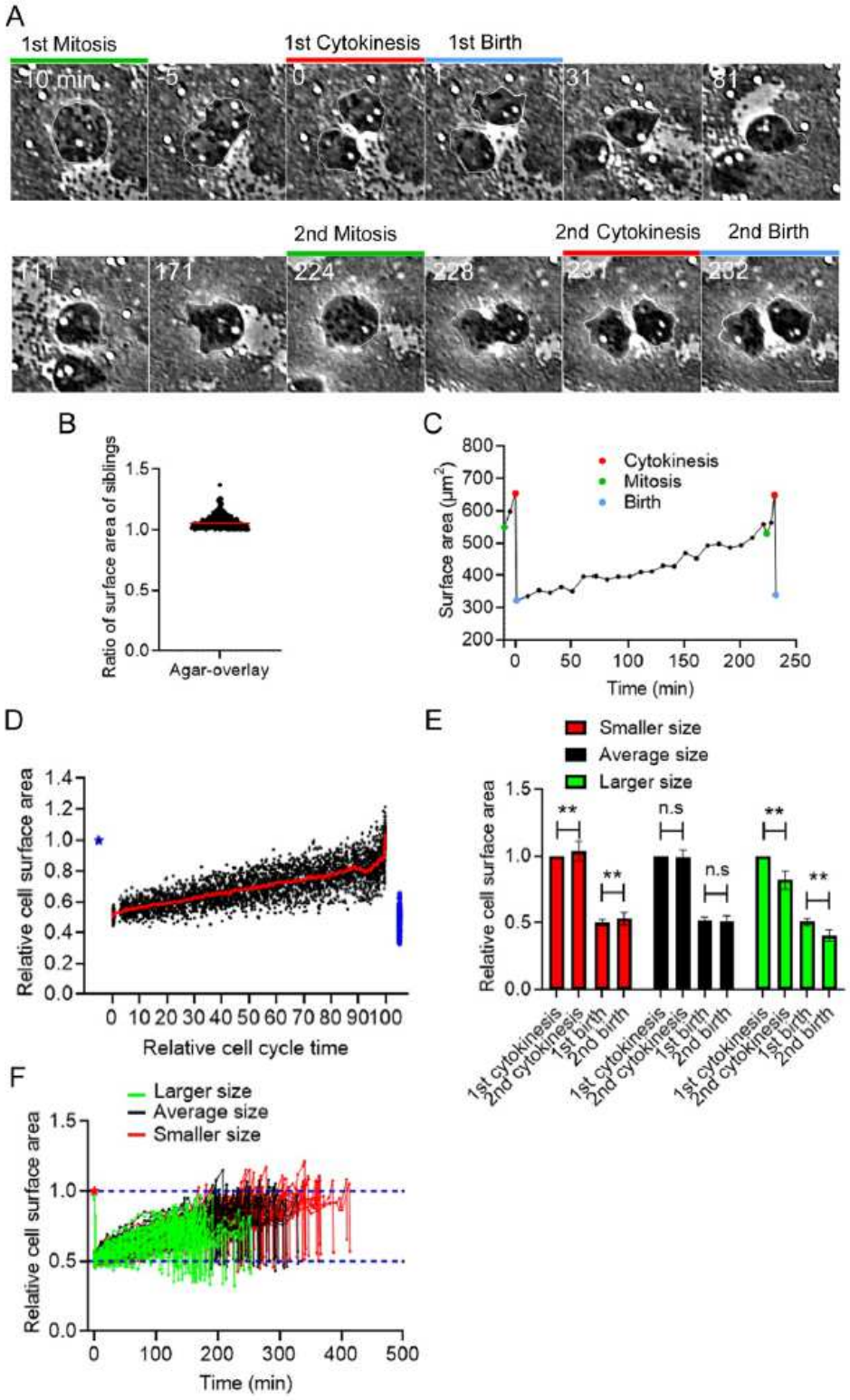


Figure 1

Control of the total cell surface area in a whole cell cycle. (A) Time course of phase-contrast images of Dictyostelium cells in whole cell cycle. The green lines show the mitotic phase, the red lines show cytokinesis, and the blue lines show the cells immediately after cell division (at birth). The cell was

outlined with white lines. Scale bar: 10 μm. (B) Differences in surface area between sibling cells. The cells divide almost evenly with a small deviation (7.02% ± 5.94%, n = 227). Data are presented as the mean ± SD. (C) Representative time course of the total cell surface area in whole cell cycle. The colored marks indicate the same events shown in Fig. 1A. (D) Time courses of the total cell surface area from the first birth (blue asterisk) to the second birth (blue points) in multiple cells (n = 147). The relative cell cycle time is normalized. (E) Comparison of the relative cell surface area between the 1st and 2nd cytokinesis and at the 1st and 2nd birth in larger (> 450 μm2, green), average (400 ± 50 μm2, black), and smaller sized cells (< 350 μm2, red). Data are presented as the mean ± SD. **P < 0.001; ns, not significant; P > 0.05. (F) Time courses of the relative cell surface area of individual cells from the first division (red asterisk) to the second division (n = 147). The blue dotted lines show 0.5 and 1.0 of the relative cell surface area, respectively.

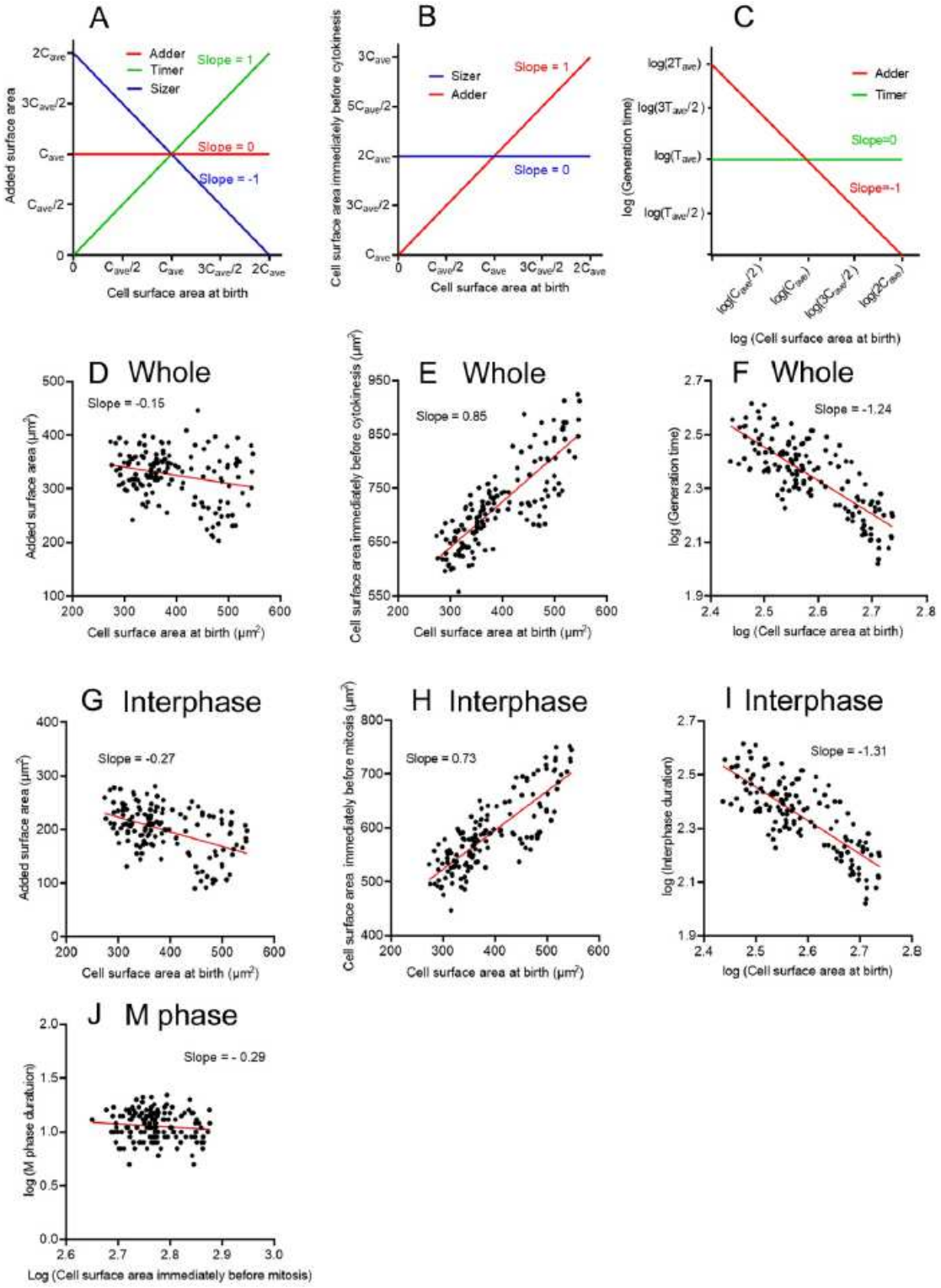


Figure 2

Cell surface area in Dictyostelium cells is regulated by near-adder model. (A-C) Ideal graphs to assess the three models to explain the regulation of cell size homeostasis. (A) The added surface area during cell cycle versus the cell surface area at birth. (B) The cell surface area immediately after cytokinesis versus the cell surface area at birth. (C) The logarithmic generation time versus the logarithmic cell surface area at birth. Cave and Tave show the average cell surface area at birth and the average generation time,

respectively. (D-F) Graphs of actual data in whole cell cycle (Whole). (G-I) Graphs of actual data between the first cell division and immediately before mitosis (Interphase). (J) A graph of actual data during cell division (M phase). Linear regression lines are shown in red.

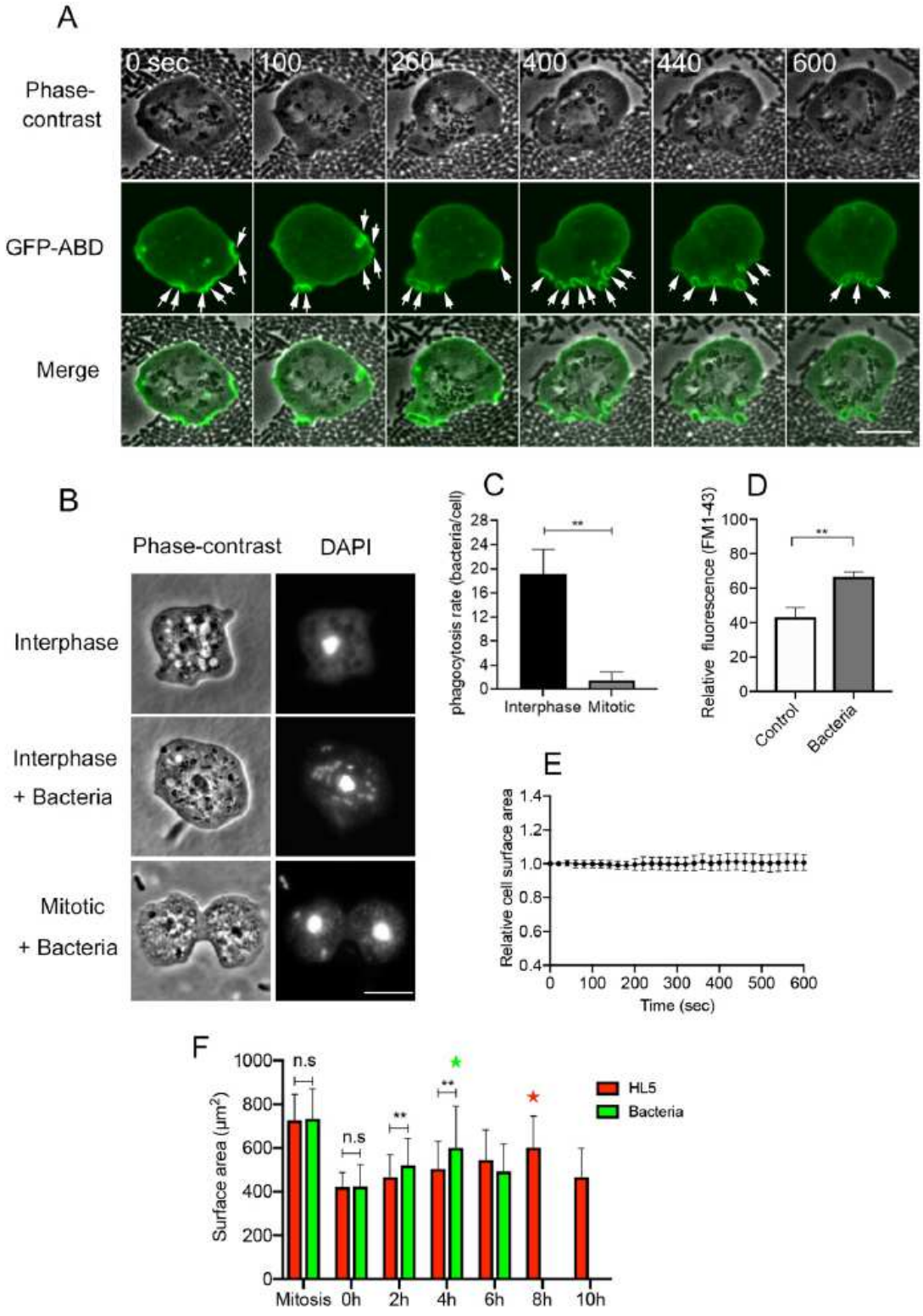


Figure 3

The cell surface area is maintained by a dynamic balance between exocytosis and endocytosis. (A) Representative time courses of phase-contrast, fluorescence, and merge images of a live cell expressing

GFP-ABD in the presence of bacteria. Arrows indicate bacteria being internalized by the cells. Scale bar, 10 μm. (B) Representative phase-contrast and fluorescence images of cells stained with DAPI in the presence and absence of bacteria. Many bacteria were found in interphase cells, but a few bacteria were found in mitotic cells. Scale bar, 10 μm. (C) The number of bacteria in cells during interphase and in mitotic cells. Data are presented as the mean ± SD. **P < 0.001; n ≥ 80 for each. (D) Quantitative analysis of the total cell surface area in the presence and absence of bacteria. Cells were stained with FM1-43 and measured using fluorescence spectrophotometry in each condition. Data are presented as the mean ± SD. **P < 0.001, three different experiments. (E) A time course of the total cell surface area in the presence of bacteria (n = 28). (F) Time courses of the cell surface area in HL5 medium and a phosphate buffer containing E.coli. Cells were arrested via cold treatment and thiabendazole (TB) at the mitotic stage (Mitosis). After releasing the mitotic arrest by removing TB, the total cell surface area was examined by using the agar-overlay method, followed by fixation at the indicated times. Data are presented as the mean ± SD and analyzed using Student's t-test. **P < 0.001; ns, not significant; P > 0.05, three different experiments.

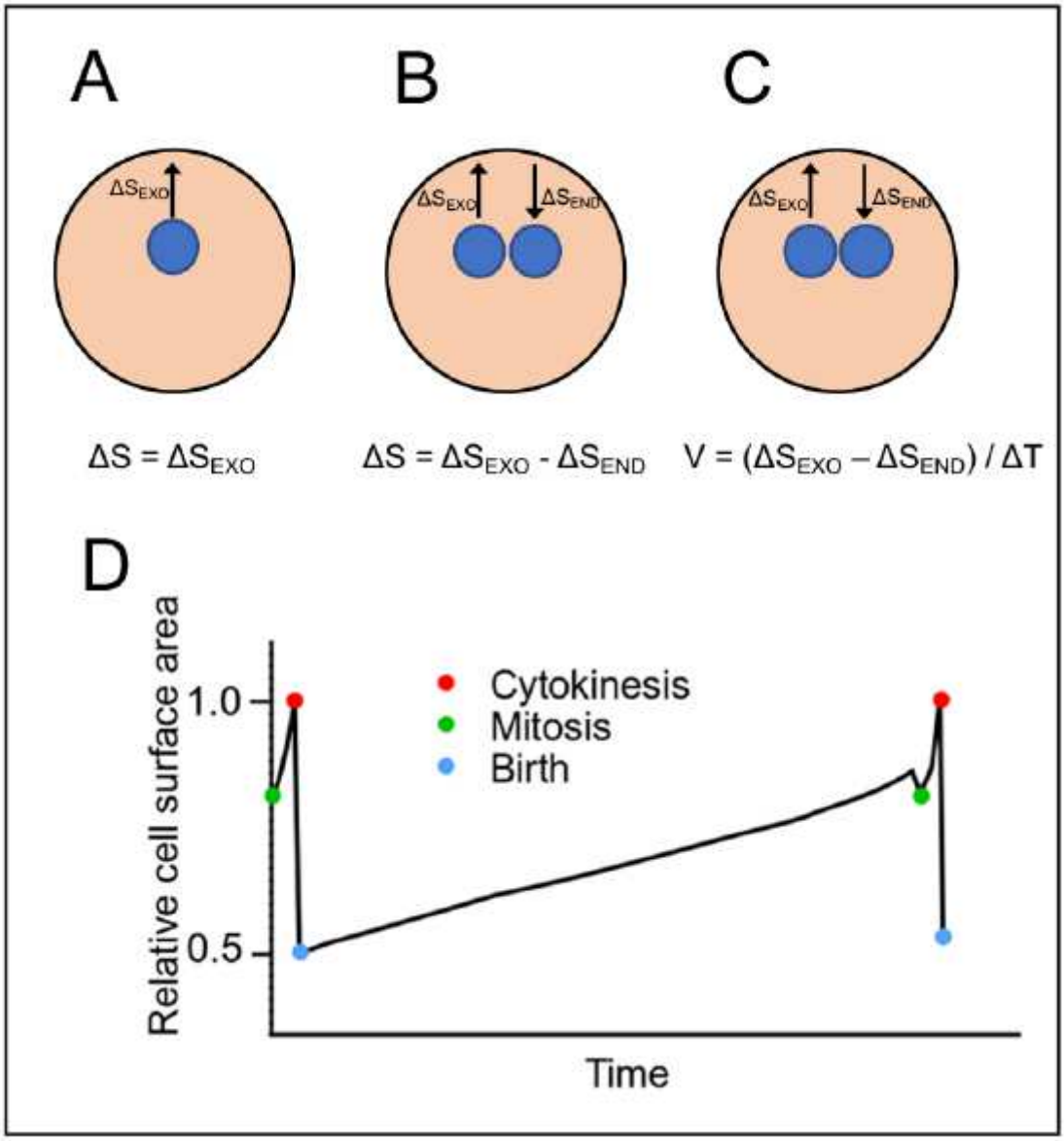


Figure 4

Dynamic adder model. (A) A previous adder model considered only the added surface area without taking into account the turnover of cell membrane. Here, ΔS is the total added surface area, and ΔSEXO is the added surface area due to exocytosis. (B) Since the cell membrane rapidly turns over, the total added surface area (ΔS) should be determined by subtracting the amount of membrane uptake by endocytosis (ΔSEND) from that of membrane supply by exocytosis (ΔSEXO). (C) The turnover rate of cell membrane (V) may regulate the cell size. V is calculated by dividing the added surface area (ΔS) by a unit of time (ΔT). (D) A summary of the dynamics of the total cell surface area in the cell cycle of Dictyostelium cells.