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Trends in Cell Biology

Opinion

Cellular mechanics during division of a genomically minimal cell

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Genomically minimal cells, such as JCVI-syn3.0 and JCVI-syn3A, offer an empowering framework to study relationships between genotype and phenotype. With a polygenic basis, the fundamental physiological process of cell division depends on multiple genes of known and unknown function in JCVI-syn3A. A physical description of cellular mechanics can further understanding of the contributions of genes to cell division in this genomically minimal context. We review current knowledge on genes in JCVI-syn3A contributing to two physical parameters relevant to cell division, namely, the surface-area-to-volume ratio and membrane curvature. This physical view of JCVI-syn3A may inform the attribution of gene functions and conserved processes in bacterial physiology, as well as whole-cell models and the engineering of synthetic cells.

Highlights

Genomically minimal cells, such as JCVI-syn3.0 and JCVI-syn3A, offer simplified systems to study conserved physiological processes.

Cell division depends on multiple genes of known and unknown function in JCVI-syn3A.

A physical description of cellular mechanics can further understanding of the contributions of genes to cell division.

Investigations of the genetic bases for key physical parameters, such as the surface-area-to-volume ratio and the membrane curvature, can clarify genotype–phenotype relationships in cell division and other processes.

Biomolecular functions of genes underlying cell division remain unknown for genomically minimal cells

Genomically minimal cells (see Glossary), such as JCVI-syn3.0 [1] and JCVI-syn3A [2,3], are emerging model systems powerfully suited to connecting cellular phenotype with genotype and environment. Based on the naturally reduced genome of the native precursor organism *Mycoplasma mycoides* subspecies *capri*, the genomes of JCVI-syn3.0 and JCVI-syn3A retain predominantly essential and quasi-essential genes for growth in rich media [1,2]. With increasingly complete annotation of gene function (GenBank: CP016816.2), JCVI-syn3.0 and JCVI-syn3A open opportunities to identify the genetic requirements for essential physiological processes. To date, research on these organisms has focused on genetic content [1], metabolism [2], genetic information processing [4], prediction of protein function [5–8], cell division and morphology [3], chromosome conformation [9], evolution [10,11], metabolite damage control [12], protein–protein interactions [13], whole-cell modeling [14], and whole-cell illustration [15]. These organisms have also served as genomically minimal platforms to study genes from other species involved in virulence [16,17] and motility [18].

Fundamental to cellular life, cell division depends on properties of the entire cell that emerge from interactions between gene products with different biomolecular functions. As mycoplasmas lack a peptidoglycan cell wall, they offer a model system for membrane studies [19] and forces acting on the membrane during cell division. Wild-type JCVI-syn1.0 has 901 genes and exhibits normal cell division resulting in cells near the resolution of widefield optical microscopy (Figure 1A). In scanning and transmission electron micrographs, JCVI-syn1.0 cells are nearly spherical and approximately 500 nm in diameter [20]. Genomically minimal JCVI-syn3.0 retains 474 of the genes in JCVI-syn1.0, and its propagation results in extreme morphological variation with many nonspherical structures larger than several microns in diameter (Figure 1B). With 492 genes, JCVI-syn3A includes 19 genes deleted in JCVI-syn3.0 and displays cells similar in appearance to JCVI-syn1.0 (Figure 1C) [3]. A reverse-genetics approach determined the genetic requirements for cell division in the genomic contexts of JCVI-syn3.0 and JCVI-syn3A [3]. In JCVI-syn3.0,

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normal cell division requires seven of the 19 genes encoding two cytoskeletal proteins known to participate in cell division, **ftsZ**/*0522* and **sepF**/*0521*, a hydrolase of unknown substrate (*0520*), and four proteins of unknown function predicted to associate with the membrane (*0527*, *0602*, *0604*, and *0605*) (Figure 1D). At least four of these seven genes – *ftsZ*, *sepF*, *0527*, and *0604* – are highly conserved in bacteria. Interestingly, deletion of *ftsZ*, *sepF*, and *0520* does not cause extreme morphological variation in JCVI-syn3A, which suggests that the genetic requirements for cell division depend on genomic context [3]. JCVI-syn3.0 retains some nonessential genes known to participate in cell division in other species, including a cytoskeletal protein, **ftsA**/*0523*, as well as *mraW*/*0524* and *mraZ*/*0525*, which regulate the expression of *ftsZ* in *Mycoplasma genitalium* [21]. Expression of *mraZ* was not detected in measurements of the JCVI-syn3A proteome [2]. Recent bioinformatic analyses identified **ezrA**/*0239* and **gpsB**/*0353* as two other genes retained in JCVI-syn3.0 known to participate in cell division in other species (D. Bianchi and Z. Luthey-Schulten, personal communication). *ezrA*/*0239* is a negative regulator of FtsZ polymerization [22], while *gpsB*/*0353* has several roles, notably in localizing proteins that contribute to cell division [23]. It remains unknown how these genes contribute to the forces driving cell division in JCVI-syn3A and related genomically minimal mycoplasmas.

A physical description of cell division can inform gene function

Providing an interlayer between genotype and phenotype, a physical description of cellular mechanics can help identify possible biomolecular functions for genes in cell division. We focus on two key parameters affecting cellular size and shape and relevant to cell division: the surface-area-to-volume ratio and the membrane curvature. Physical models of shape transformations in vesicles may offer initial descriptions of cellular shape as a function of these parameters (Box 1). We review current knowledge on which genes may contribute to these parameters, to guide future measurements and experiments.

Genes affecting the surface-area-to-volume ratio during division are likely to include those involved in regulating osmotic pressure and incorporating lipids

Although many genes contribute to cellular growth, we focus here on genes that may directly affect volume growth by regulating the **osmotic pressure gradient** across the membrane or surface area growth by regulating the incorporation of lipids.

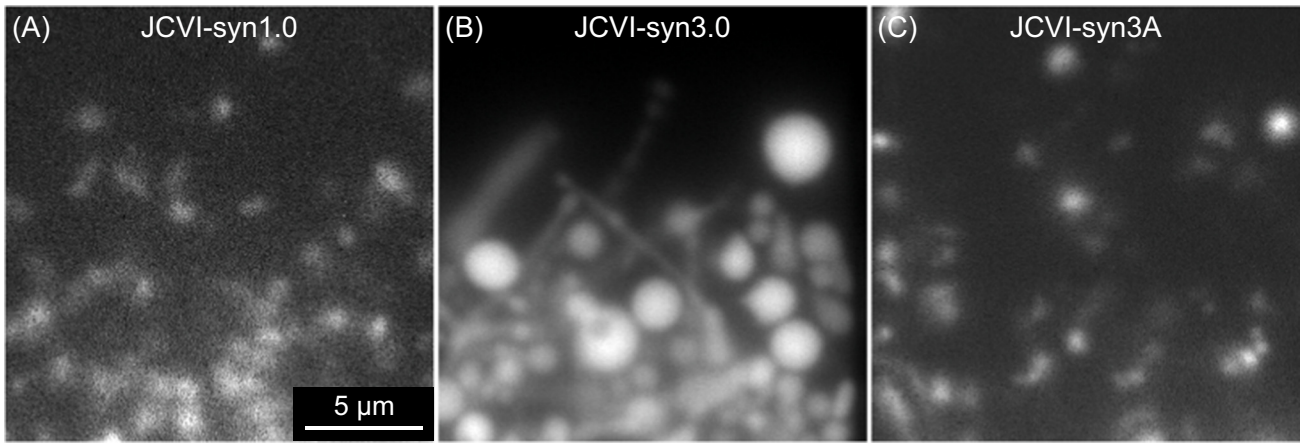
The majority of volume growth may be driven by the flow of water into the cell, as water accounts for approximately 65% of the intracellular mass of the native precursor organism *M. mycoides* [24]. JCVI-syn3A retains 30 transporters composed of 53 proteins, some of which could contribute to osmoregulation [25–28]. JCVI-syn3A retains the potassium transporter regulator **trkA**/*0686*, which can respond to hyperosmotic shifts [26] (Figure 2A). The associated potassium channel *trkG* or *trkH* has not been annotated in JCVI-syn3A. At least 13 transporters comprising 22 proteins remain unassigned in JCVI-syn3A (*0030*, *0034*, *0165*, *0166*, *0167*, *0168*, *0169*, *0195*, *0196*, *0197*, *0325*, *0399*, *0639*, *0696*, *0797*, *0822*, *0870*, *0876*, *0877*, *0878*, *0879*, *0881*) (J. Glass, personal communication). In addition to these 22 proteins, JCVI-syn3.0 retains the unassigned transporter *0531*, absent in JCVI-syn3A. Unidentified transporter substrates may include choline, which modulates intracellular potassium in *M. mycoides* [29] and contributes to osmoregulation in other species [26]. *M. mycoides* grows in media over a range of osmolarities from approximately 0.3× to 1.8× isotonic saline [30] and survives lysis down to approximately 0.1× isotonic saline [31]; however, JCVI-syn3.0 and JCVI-syn3A may grow and survive over a narrower range of osmolarities as they lack the aquaglyceroporin *glpF*/*0217*, which conducts water [32] and is present in JCVI-syn1.0. It also remains unknown in JCVI-syn3A how regulation of the osmotic pressure couples to the global translation rate. As one possible link between intracellular metabolite concentrations and translation rates, we note that JCVI-syn3A retains **relA**/*0414*, which synthesizes the growth inhibitor (p)ppGpp in response to nutrient limitation [33].

Glossary

Physics glossary:
Bending rigidity, κ: describes the stiffness of a membrane in response to bending deformations.
Dimensionless spontaneous curvature, $\bar{m}$: m normalized by the M of a sphere with the same surface area.
Mean curvature, $M(s_1,s_2)$: at point (s_1,s_2) on the membrane surface, consider the normal vector $\hat{n}$, then consider the space of planes that contain $\hat{n}$. The intersection of any plane with the membrane surface defines a curve. M equals the average of the maximum and minimum curvatures of these curves.
Osmotic pressure gradient: osmotic pressure inside minus outside the cell.
Reduced volume, v: a dimensionless metric for the surface-area-to-volume ratio, equal to the actual volume divided by the maximum volume allowed by the actual surface area.
Spontaneous curvature, m: M corresponding to zero bending energy.
Biology glossary:
Cardiolipin: lipid with a conical shape that enriches in regions of higher curvature.
Cholesterol: lipid that rapidly flip-flops between leaflets.
ClsA: protein that synthesizes cardiolipin.
EzrA: protein that negatively regulates FtsZ polymerization.
Flippase: protein that hydrolyzes ATP to translocate lipids from the outer leaflet to the inner leaflet.
Floppase: protein that hydrolyzes ATP to translocate lipids from the inner leaflet to the outer leaflet.
FtsA, SepF: proteins that interact with FtsZ and participate in cell division.
FtsZ: protein that polymerizes into filaments that often localize at the division site and contribute to cell division.
Genomically minimal cell: synthetic cell with a genome that retains predominantly essential genes.
GpsB: protein that localizes other proteins contributing to cell division.
Lipoproteins: proteins tethered to the outer leaflet of the membrane by covalent modification with fatty acid chains.
RelA: protein that synthesizes the growth inhibitor (p)ppGpp in response to nutrient limitation.
Synthetic cell: here, a cell constructed by genome transplantation of a genome

Surface-area growth results from the addition of membrane components. The membrane of JCVI-syn3A comprises approximately half protein and half lipid [14], and a small number of lipid types account for a significant fraction of the surface area. We consider genes contributing to the incorporation of lipids from the growth medium and the transfer of lipids between leaflets. In the related species *Mycoplasma capricolum*, fatty acid incorporation is active and mediated by protein [34], but genes contributing to lipid incorporation have not been determined in JCVI-syn3A. Genes *0371* and *0372* are annotated as *ywjA* **flippases**, which translocate lipids from the outer leaflet to the inner leaflet (Figure 2B). The annotation of gene *0639* was recently changed to a possible FtsX-like permease, a family of proteins associated with **floppase** activity, transporting lipids from the inner leaflet to the outer leaflet. We note that the lipid composition of the growth medium can affect cell shape in other *Mycoplasma* species [19], and similar effects have not yet been explored in JCVI-syn3A.

assembled from oligonucleotides into a naturally evolved recipient cell.
TrkA: subunit of a membrane transporter that hydrolyzes ATP to import potassium into the cell.



(D) Retained in JCVI-syn3A but not JCVI-syn3.0 Retained in both JCVI-syn3A and JCVI-syn3.0

Locus tag	Annotation	Biomolecular function	Proteins/cell*
0239	EzrA	Division apparatus	43±8
0353	GpsB	Division apparatus	92±20
0520	Alpha/beta hydrolase superfamily	Unknown	25±6
0521	SepF	Division apparatus	59±7
0522	FtsZ	Division apparatus	646±75
0523	FtsA	Division apparatus	40±7
0524	RsmH/MraW	RNA methyltransferase	32±2
0525	MraZ	Transcription factor	Not reported
0527	Protein of unknown function, DUF177 domain	Unknown	256±61
0602	Protein of unknown function	Unknown	Not reported
0604	LemA/GacS family	Unknown	361±16
0605	Protein of unknown function	Unknown	86±16

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Figure 1. Several genes required for cell division in a genomically minimal cell do not have a known biomolecular function. (A–C) Optical micrographs show cells with constitutively expressed mCherry to label the cytoplasm, prepared during an experiment using a microfluidic chemostat to culture and image cells [3]. (A) Wild-type JCVI-syn1.0 exhibits cells near the resolution of widefield optical microscopy that diffuse throughout the 3-μm depth of the chemostat. (B) Genomically minimal JCVI-syn3.0 exhibits extreme morphological variation. (C) JCVI-syn3A includes 19 genes not retained in JCVI-syn3.0 and appears similar to JCVI-syn1.0. (D) Blue: Seven of the 19 genes are required together to reduce morphological variation in the genomic context of JCVI-syn3.0. Five of these seven genes have no known biomolecular function. Gray: The JCVI-syn3.0 genome retains some genes known to participate in cell division, including *ezrA/0239, gpsB/0353*, *ftsA/0523*, *mraW/0524*, and *mraZ/0525*. *Averages and standard deviations calculated from triplicate proteomic measurements of JCVI-syn3A reported in [2].

Box 1. A simple model for vesicles may approximate cellular shape and mechanics in genomically minimal cells

A model for shape transformations in vesicles may guide the first efforts to conceptualize cell division in genomically minimal cells. Mycoplasmas have a fluid membrane and lack a peptidoglycan cell wall [19] and JCVI-syn3A cells exhibit dynamic shape fluctuations qualitatively consistent with a fluid membrane [3]. We consider here the spontaneous-curvature (SC) model [47–50], which assumes that the area difference between membrane leaflets may change by the flip-flopping of at least one component. This assumption may apply to JCVI-syn3A, because **cholesterol** accounts for approximately 60% of JCVI-syn3A lipids by number [14], and cholesterol rapidly diffuses from one leaflet to another with a leaflet residence time of 10 ms in vesicles [51]. The SC model assumes a uniform distribution of curvature-inducing molecules over the vesicle surface and vesicle shapes with a minimized membrane bending energy. These assumptions may not apply to JCVI-syn3A, due to the unknown distribution of curvature-inducing molecules over the cellular surface and the unknown effects of these molecules on cellular shape. Although the SC model ignores such effects, its phase diagram includes partially and fully constricted shapes (Figure IA) akin to dividing cells. Some trajectories through the phase diagram represent cycles, for example, in which a single sphere elongates and constricts to become two spheres (Figure IB), analogous to replication cycles.

The SC model has only two independent parameters: the **reduced volume** v and the **spontaneous curvature**, m [47–49]. v parameterizes the surface-area-to-volume ratio and equals the actual volume divided by the maximum volume allowed by the surface area, corresponding to a fully inflated sphere with radius $R_0 = \sqrt{A/4\pi}$. A single sphere has $v = 1$ and two spheres connected by an infinitesimal tube have $v = 1/\sqrt{2}$ (A). m represents the membrane curvature with zero bending energy, with a positive sign conventional for curving toward the inside of the vesicle. A **dimensionless spontaneous curvature**, $\overline{m} = m \cdot R_0$, normalizes m by the curvature of a sphere with radius R_0, such that $\overline{m}$ does not depend on vesicle size. For any physical pair of (v,m), the SC model calculates the shape of the minimum bending energy $H = 2\kappa \oint dA\,(M(s_1,s_2) - m)^2$ integrated over the vesicle surface, with κ the **bending rigidity** and M the **mean curvature** at surface coordinate (s_1,s_2).

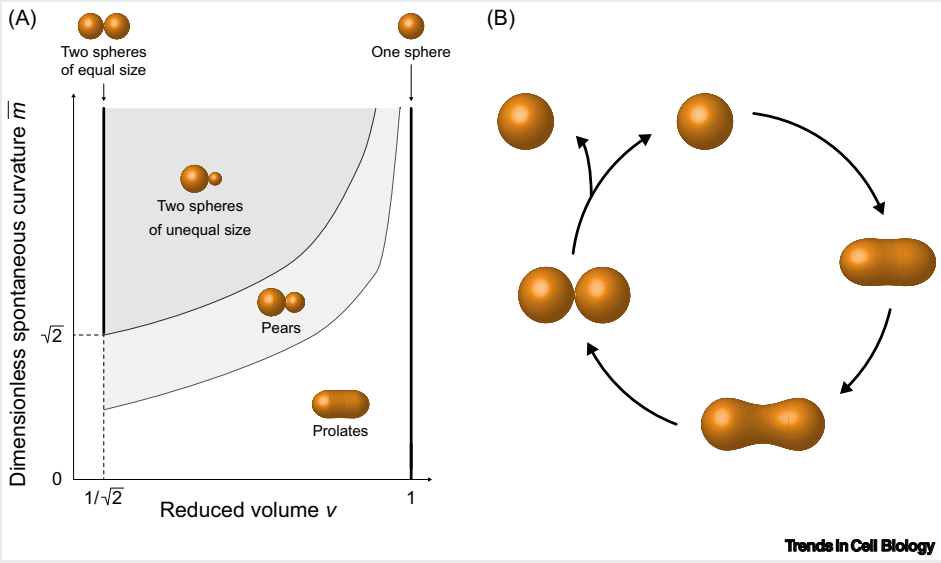

Figure I. The spontaneous-curvature (SC) model for vesicles predicts constricted shapes at sufficiently high surface-area-to-volume ratio and membrane curvature. (A) A region of the phase diagram for the SC model. Adapted from [48,49]. Shapes illustrate the qualitative features of each region of the phase diagram. (B) The SC model describes shape trajectories in which a spherical vesicle grows and constricts to yield two spherical vesicles, representing a possible shape trajectory over a cellular replication cycle. Adapted from [50].

Genes affecting membrane curvature during division are likely to encode curvature-inducing molecules with nonuniform distributions

Cell division requires changes in membrane curvature, resulting from the intrinsic biophysical properties of membranes acted upon by gene products. The simple spontaneous-curvature (SC) model for shape transformations in vesicles describes spontaneous constriction for sufficiently high excess surface area and membrane curvature (Box 1). In JCVI-syn3A, gene products

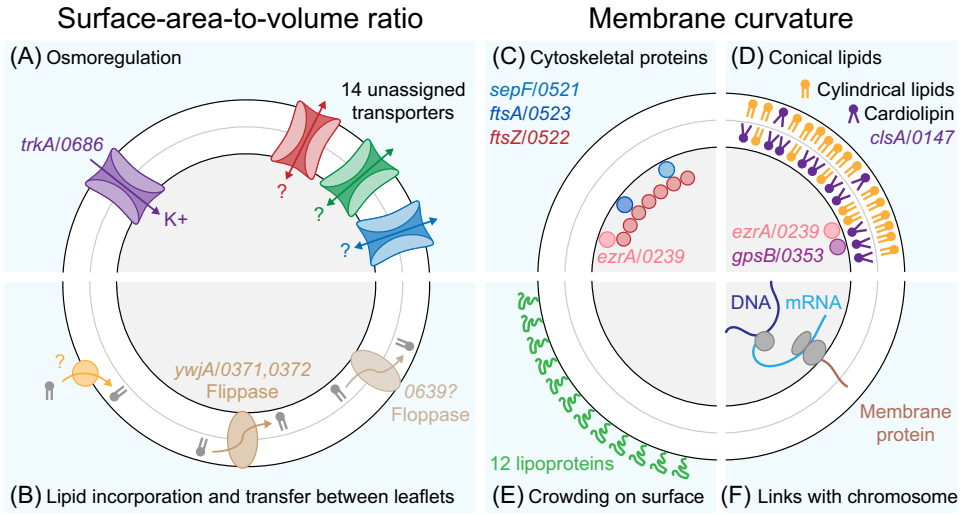

Figure 2. Genes likely to contribute to the surface-area-to-volume ratio and membrane curvature in JCVI-syn3A. These examples are chosen based on our knowledge on the genome. Other genes may also contribute to these properties. Question marks in (A) represent the unknown substrates and directionality of 14 unassigned transporters, and in (B) represent unknown genes contributing to lipid incorporation from the growth medium. The lipid specificity of the gene products in (B) is unknown.

may not only generate curvature but also implement spatiotemporal control, for example, to coordinate cell division with cell growth and chromosome segregation.

Based on current gene annotations, curvature-inducing processes that may contribute to cell division in JCVI-syn3A include: bending by intrinsically curved cytoskeletal filaments, such as FtsZ, associated with the inner leaflet of the membrane (Figure 2C); nonuniform distribution of the conical lipid **cardiolipin** (Figure 2D); crowding of **lipoproteins** in the outer leaflet of the membrane (Figure 2E); and interaction between the membrane and chromosome (Figure 2F).

FtsZ forms intrinsically curved polymers [35] that can deform and constrict vesicles, when anchored to the membrane directly via an engineered tether [36,37]. JCVI-syn3A retains several gene products known to interact with FtsZ and participate in cell division, including *sepF/0521* and *ftsA/0523*. Degradation of FtsZ affects the timing of cell division in starved or slow-growing *Escherichia coli* [38,39], and JCVI-syn3A retains the protease *lon/0394*, which degrades FtsZ in *Mycoplasma pneumoniae* [40]. The importance of FtsZ for cell division in JCVI-syn3A remains unclear. For example, recent laboratory-evolution experiments suggest that an *ftsZ* E315* truncation mutant increases fitness and does not significantly affect cellular size [11]. The C-terminal tail of FtsZ, truncated in this mutant, affects many aspects of FtsZ function including polymerization, lateral association of polymers, and interaction with other proteins [41].

The conical lipid cardiolipin may contribute to membrane curvature during cell division. Cardiolipin synthase **clsA***/0147* is essential in JCVI-syn3A [2] and cardiolipin accounts for approximately 15% of JCVI-syn3A lipids by number [14]. These lipids partition into regions of higher curvature in vesicles [42] and increase the binding affinity of supported lipid bilayers to DivIVA, a homolog of GpsB (*0353*) [43]. Consistent with additional roles for GpsB outside cell division [23], *gpsB/0353* is quasi-essential in JCVI-syn3A [2]. Transfer of lipids between leaflets by *ywjA* flippases

(*0371* and *0372*) and possible *ftsX*-like floppase (*0639*) (Figure 2B) may also contribute to membrane curvature via the difference in surface area between leaflets.

Crowding of membrane proteins or polymers in the outer leaflet can drive the division of vesicles [44,45]. In vesicles, a surface density of approximately 150 GFP/μm^2 induces a spontaneous curvature of approximately 1/(250 nm) [45], comparable with the radius of a JCVI-syn3A cell [9]. JCVI-syn3A retains 12 uncharacterized lipoproteins localized in the outer leaflet (*0108*, *0338*, *0398*, *0439*, *0440*, *0481*, *0505*, *0602*, *0605*, *0622*, *0636*, *0835*, *0851*) [2], including *0602* and *0605* required for normal cell division [3]. Based on proteomic measurements of JCVI-syn3A [2], we estimate the total surface density of these lipoproteins is approximately 3000 lipoproteins/μm^2. As in vesicles, crowding of lipoproteins may generate significant curvature in JCVI-syn3A.

Chromosome mechanics may contribute to cellular shape and division in JCVI-syn3A, because the energy to deform the chromosome may be comparable with the energy to deform the membrane. The chromosome may link to the membrane via transertion, which is the coupled transcription, translation, and insertion of membrane proteins [46]. We note that approximately 20% of ribosomes were observed near the membrane in cryoelectron micrographs [9].

Concluding remarks

Measuring the size, shape, and mechanical properties of JCVI-syn3A cells would further our understanding of how genotype gives rise to phenotype in this minimal, synthetic organism (see Outstanding questions). However, the small size, photosensitivity, and deformability of JCVI-syn3A cells present challenges to current measurement techniques. The diameter of JCVI-syn3A cells ranges from 400 to 500 nm in cryoelectron micrographs [9]. Thus, measuring cellular shape and localizing intracellular gene products requires a spatial resolution of tens of nanometers or less. The small size of the cells complicates direct *in situ* measurements of forces generated by curvature-inducing molecules. Cells can incur significant growth defects due to phototoxicity, and mitigation of phototoxicity would help measurement of the dynamics of cellular shape over the cell cycle. Synchronization of cells has not been achieved and would also enable omics characterization of cells over the cell cycle. *Mycoplasma* cells can deform in shear flow as they lack a cell wall. Confinement of cells to fluidic chambers with dimensions comparable with individual cells may facilitate measurement but perturb cellular shape.

Given the genetic tractability of genomically minimal cells, engineering the cells themselves may render them more amenable to measurement. Understanding the genetic basis for cellular size could inform whole-genome engineering to increase cellular size. Engineering cellular metabolism could help increase resistance to oxidative damage. Functionalizing the cellular surface could increase stability during liquid handling or facilitate the immobilization of cells.

As the simplest known cell, JCVI-syn3A offers an empowering framework to apply a physical description of cellular mechanics to attribute biomolecular functions to genes and understand how genotype gives rise to phenotype. The resulting insights may guide the engineering of division and self-replication in **synthetic cells**, as well as the maintenance of mechanical integrity in differing environments. With less control of cell division and morphology than JCVI-syn3A, JCVI-syn3.0 may serve as a model for ancestral cells, for example, to study how physiological processes can emerge from the intrinsic biophysical properties of lipid membranes, DNA polymers, and the cytoplasm as a colloidal system. This physical approach can extend from cell division to more general aspects of cell structure and physiology, such as the organization of the cytoplasm and genome over the cell cycle.

Outstanding questions

What are the biomolecular functions of the genes of unknown function required for cell division in JCVI-syn3A?

How can simple physical models help to attribute function to the remaining genes of unknown function in genomically minimal cells?

How do the size, shape, and mechanical properties of JCVI-syn3A depend on gene content, gene expression, and growth conditions?

What measurement techniques and experimental protocols are compatible with the small size, photosensitivity, and deformability of JCVI-syn3A?

How can genomically minimal cells be engineered to be more amenable to measurement?

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Declaration of interests

The authors declare no interests.

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