FEBS Letters 589 (2015) 2726–2730

FEBS Letters

journal homepage: www.FEBSLetters.org

E. coli MG1655 modulates its phospholipid composition through the cell cycle

Samuel Furse [a,*], Hans Wienk [b], Rolf Boelens [b], Anton I.P.M. de Kroon [a], J. Antoinette Killian [a]

[a] Membrane Biochemistry and Biophysics, Department of Chemistry, Universiteit Utrecht, Kruytgebouw, Padualaan 8, 3584 CH Utrecht, The Netherlands
[b] NMR Spectroscopy, Bijvoet Center for Biomolecular Research, Department of Chemistry, Universiteit Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 22 May 2015
Revised 7 July 2015
Accepted 28 July 2015
Available online 10 August 2015

Edited by A. Chattopadhyay

Keywords:
Lipid
Lipidomic
Cell cycle
Cell cycle control
Lipid metabolism

ABSTRACT

This paper describes a study of the phospholipid profile of *Escherichia coli* MG1655 cultures at the B and D periods of the cell cycle. The results indicate that the phosphatidyl glycerol fraction grows relatively rapidly and that the size of the cardiolipin (CL) fraction does not grow at all during cell elongation. This is consistent with observations that CL is located preferentially at the poles of *E. coli*. It also suggests that lipid production is controlled as a function of the cell cycle.

1. Introduction

How cells grow and divide has been a central question in biology since they were discovered. In bacteria, the cell cycle is conventionally divided into three periods [1–4]. First, the phase between division and the initiation of replication (B period). Second, the period in which the nucleolus is replicated and in which the bacterium reaches its mature size of ~2 μm (C period, also called the replication phase). Third, the period between replication of the DNA and division of the cell envelope, during which the cell elongates to its greatest extent (D period). Despite increasing research efforts in recent decades, it is still not clear which factors control bacterial cell division. Understanding this process is useful for combatting bacterial infections and for the use of bacterial cultures in industrial preparations of organic molecules.

Over the last 40 years, inspiring research into the cell cycle of eukaryotic systems has shown that cyclin and cyclin-dependent kinases are key components in the control of the cell cycle in higher organisms [5–7]. However, no similar proteins have yet been found in bacteria or archaea [8]. Furthermore, there is no evidence for regulation by genetic [9] or structural protein [10,11] means in bacteria. It is therefore conceivable that it is the expansion and shape of the cell envelope that has the dominant influence over controlling binary fission in bacteria. If correct, one would expect that the physical properties of the lipids to be important in cell division. As a result, shifts in the lipid profile would occur as a function of the cell cycle in order to minimise the energetic cost of a membrane-controlled cell division.

The physical properties of lipids that may be of particular importance for the division process are the intrinsic curvature and the charge of the lipids. Two bacterial lipids, phosphatidylethanolamine (PE) [12] and cardiolipin (CL) in the presence of divalent cations [13,14] have been identified as lipids that can induce negative (type II) curvature. These lipids may be necessary for ensuring that the membrane itself curves in the proper way for the stage of division the cell is in or is preparing for. Indeed, there is evidence that the presence of lipids with a negative intrinsic curvature can lower the energetic barrier to changes in membrane curvature consistent with membrane fission intermediates [15–17]. More specific evidence about the importance of lipids for cell division in bacteria relates to the interactions of negatively charged lipids with other biomolecules. Anionic lipids are required for assembly of the 'Z-ring', the protein aggregation that is essential for binary fission [18], for proteins that regulate the formation of the division plane [19] and for DNA

Author contributions: S.F. conceived the research questions, carried out the experiments, analysed and interpreted the data. S.F. and J.A.K. wrote the manuscript. H.W. and R.B. made instruments available, optimised acquisition parameters and assisted in acquiring NMR data. A.I.P.M.d.K. and J.A.K. wrote the original grant application. All authors commented on the manuscript during preparation and gave approval to the final version.

* Corresponding author.
E-mail addresses: s.r.furse@uu.nl, samuel@samuelfurse.com (S. Furse).

http://dx.doi.org/10.1016/j.febslet.2015.07.043
0014-5793/

replication [20–22]. However, as the various processes in cell division only occur at certain points of the cell cycle, the physical properties of the lipids that are involved would be required only at these particular points in the cycle. It would therefore be necessary for the cell membrane to be inhomogeneous either with respect to spatial arrangement (*i.e.* locus of the lipids) or time (*i.e.* change through the cell cycle), or both.

Several recent microscopy studies provide clear evidence that lipid distribution is inhomogeneous with respect to spatial arrangement. This work focussed mainly on the principal anionic phospholipid species, phosphatidylglycerol (PG) and CL [23,24]. Specifically, epifluorescence experiments showed the formation of micro-domains at highly curved structures in spheroplasts, consistent with CL being concentrated at the poles [24]. Studies using the anionic-lipid-binding dye 10-*N*-nonyl acridine amongst other techniques suggested that both CL and PG may be found at the poles [23] and that CL may also be found at the septal regions [23,25].

In order to determine whether the lipid profile is inhomogeneous with respect to time, and thus the cell cycle, different approaches are required. The lipid fraction of the highly mutated *Escherichia coli* B/r strains has been explored as a function of the cell cycle [26–29], with approaches either being based upon synchronising cultures in a timed fashion (cell age) [26–28], or separating cells by mass using centrifugation (cell size) [29]. However, these studies provided contradictory evidence for changes in the lipid profile. For example, there is evidence for both a decreasing [26,27] and an approximately static [28] phosphatidyl glycerol (PG) fraction during the cell cycle, as well as a slight increase in the concentration of PG at the mid-point of the cycle (measured by cell size, cells in the B period) but at no other point [29]. Also, there is evidence for a cyclical change in phosphatidylethanolamine (PE) concentration as a function of time, though it is not clear whether CL follows the same pattern [28]. This variety of results raises the question of whether a parent, wild-type (WT) *E. coli* strain may offer a clearer insight into lipid metabolism as a function of the cell cycle. To date, there has been no published data on the phospholipid profile of any WT *E. coli* strain at a verified stage of the bacterial cell cycle for either global or spatial lipid profiling.

In this study, we use *E. coli*, MG1655, a commonplace K12 WT approximation [30]. Cultures were stopped after elongation but before cell division (approximately at the D period) using the bacteriostatic rifampicin (RIF) in a modification of a published method [31–34] and lipid fractions of these and control cultures were profiled using high-resolution solution-phase ^{31}P NMR [35–38]. ^{31}P NMR was adopted as the technique of choice because it allows non-destructive, unambiguous, high-resolution, quantitative measurement of all phosphorus-containing molecular species present, and thus all phospholipids. Cell imaging using light microscopy was used to establish the length of the cells and to verify whether or not cultures were elongated in a manner consistent with the D period.

The results of this study show that there is a distinct difference in the proportions of the principal phospholipids as a function of the cell cycle. The possible implications of these findings in terms of the control of bacterial cell cycle are discussed.

2. Materials and methods

2.1. Reagents

Chemicals were purchased from *Sigma Aldrich* (Dorset, Gillingham, UK) and used without purification. RIF was stored at 4 °C. PhosSTOP tablets were purchased from *Roche* (Welwyn, Hertfordshire, UK) and stored at 4 °C.

2.2. Optimisation of culture growth

Cell growth was optimised by the addition of both glucose (1 g/L) and phosphate (50 mg/L) (Supplementary Fig. 1A) to commercially available LB medium (final concentration of NaCl 10 g/L, *Sigma Aldrich*). The addition of phosphate avoids the effects of low phosphate availability on the lipidome [39,40] and glucose provides a respiratory carbon source. The shift in *p*H associated with glucose metabolism (Supplementary Fig. 1B) does not appear to hinder growth.

2.3. Synchronisation and harvest of cultures

The preparation of one set of cultures (n = 5 sets used in total) is described. Several cultures of each type were grown in each set in order to ensure that enough material was produced and to account for otherwise significant differences between populations. Mini-cultures (12 × 10 mL, optimised LB) were incubated (16 h, 37 °C) before inoculating 12 × 1 L optimised LB broths to give cultures of OD_{600} ~0.03 that were incubated at 37 °C. After 2 h (average OD_{600} 1.02), three cultures were mixed and harvested (fixed-angle rotor, 9k × *g*, 4 min, 4 °C) together (making the 2 h control time point), three cultures were treated with RIF (150 mg/L final concentration, added as methanolic solution of, 15 mg/mL), and two cultures were treated with methanol (1% *v*/*v*, final concentration). After 6 h, two control cultures were mixed and harvested together (making the 6 h control time point), the two cultures treated with methanol were mixed and harvested together (making the 6 h time point with 1% methanol) and the three cultures treated with rifampicin were mixed and harvested together. After 12 h, the remaining two control cultures (12 h) were mixed and harvested together. The data for cells in the D period after 6 h growth were obtained by growing cultures as above for 6 h before administering RIF (150 mg/L final concentration, as above) and incubation (4 h) before mixing and harvesting. In contrast to previous studies, the protein synthesis inhibitor chloramphenicol (CAP) was not used [31–33]. Cell pellets were resuspended (distilled H_2O, total volume of 25 mL) including PhosSTOP (1 tab/25 mL final concentration) and 2-butoxyphenylboronic acid (1 mg/mL final concentration, ethanolic stock solution 100 mg/mL) [37,41]. Cell suspensions were then frozen ($N_{2(l)}$) and freeze-dried. The dried material was stored at −20 °C until isolation of the lipidome.

2.4. Microscopy

Cells were photographed after harvesting and before the administration of lipase inhibitors. Images of the cells were used to characterise the cultures and measure the length of cells. Images were taken using a Nikon D-eclipse C1 microscope equipped with a DXM1200 camera and processed with EZ-C1 2.20 software. Cell length was determined by measuring the lengths of 100 cells each from at least two images, for each culture/time point, with the mean and standard deviation calculated for each.

2.5. Isolation of the lipid fraction

The original Bligh and Dyer isolation [42] was adapted [43] for this study: freeze-dried cell matter was resuspended in distilled H_2O (50 mL), diluted with a mixture of chloroform (50 mL), methanol (115 mL) and $HCl_{(aq)}$ (6 M, 825 μL) before being agitated (5 min) and allowed to stand (45 min). The homogeneous mixture was made biphasic by the addition of chloroform (50 mL), $NaCl_{(aq)}$ (3·07 M/half-saturated brine, 50 mL) and HCl (6 M, 825 μL). This biphasic mixture was agitated (5 min) before filtration (glass sinter) to remove the precipitate. The aqueous fraction(s) were

washed with chloroform (50 mL) and the organic solutions combined. The latter were washed (salt buffer (EDTA, 100 mM; NaCl, 100 mM; Tris, 50 mM; *p*H 8.2), 50 mL) and the solvent removed *in vacuo*.

2.6. Phospholipid profiling by ^{31}P NMR

Samples consisted of the relevant phospholipid fraction (15–18 mg) dissolved in the (phosphorus-free) CUBO solvent system [35–38] (500 μL). Signals were identified using established chemical shift assignments [35–38]. Data acquisition was similar to published work [37] but using a Bruker 750 MHz Avance spectrometer equipped with a 5 mm QXI probe operating at 298 K. Spectra were acquired using inverse gated proton decoupling, with 4–8 k scans per sample and a spectral width of 24.95 ppm. An overall recovery delay of 8.4 s was used which gave full relaxation. Data were processed using an exponential line broadening window function of 1.50 Hz prior to zero filling to 32 768 points, Fourier transform and automatic baseline correction. Spectra were processed and analysed using (the deconvolution function in) TopSpin 2.1.

3. Results

The lipid profile of *E. coli* MG1655 in the B and D periods and thus as a function of the cell cycle was measured by sampling from cultures grown under control conditions. The initial cultures were unsynchronised. Light microscopy collected at 100× magnification (Fig. 1A and C) showed that >80% of the cells were in the B period, based on their size distribution. The effectiveness of rifampicin in holding cultures at the end of the C period/start of the D period was also demonstrated using imaging data (100× magnification, Fig. 1B). Cultures identified by microscopy as being in the D period comprised cells that were approximately 80% longer than those of unsynchronised cultures (Table 1, line 1). The modified synchronisation technique used (developed from several that employed rifampicin [31–33]) did not lead to an increase in optical density (OD, Table 1, line 2) after further incubation, suggesting that the drug acts rapidly. No change in cell width or other morphology was observed, suggesting that comparisons between treated and untreated cultures reflect the changes involved in cell elongation (normal D period activity) only.

The lipid fraction was extracted from freeze-dried cultures using a modified Bligh & Dyer isolation, that ensures that inorganic phosphate is not isolated with the phospholipids. The size of the CL, phosphatidic acid (PA), PE and PG fractions was then measured using solution phase ^{31}P NMR. A phosphorus-free solvent system designed to dissolve phospholipids to high concentrations and *mono*-dispersed, but no inorganic phosphate was used to prepare samples [35–38]. The signal areas of ^{31}P NMR spectral resonances (example spectra are shown in Fig. 2) were used to determine the relative size of the fractions of CL, PA, PE and PG (Table 1, lines 3–6). The lipid profiles of the control cultures (2 h, 6 h, Table 1 and Supplementary Fig. 2) are broadly consistent with earlier studies of WT *E. coli* cultures, with ⩾80% PE and the remaining fraction comprising PG and CL [44], and with the observation of increasing CL fraction with the age of the culture [43,44].

Table 1
Characterisation of the lipid fractions isolated from *E. coli* MG1655 cultures. Line 1, mean cell length measured by light microscopy (see also Fig. 1); line 2, Optical density at harvest measured using a single-beam spectrophotometer; lines 3–6, phospholipid fractions determined by ^{31}P NMR, after isolation using a modified version of the Bligh and Dyer method. NMR data are presented as the percentage integration of the mole fraction (%) and represent the mean and standard deviation of $n = 5$ sets. Trace amounts of PS and lyso-PG were detected in some samples.

		Incubation time/cond.		
		2 h	2 h (D period)[a]	6 h[b]
1	Cell length (mean, μm)	2.2 ± 0.3	3.6 ± 0.5	1.8 ± 0.7
2	OD_{600} at harvest	1.0 ± 0.1	1.0 ± 0.1	2.6 ± 0.3
3	PA (mean, %)	0.9 ± 0.3	0.4 ± 0.2	0.8 ± 0.3
4	PG (mean, %)	4.1 ± 1.0	6.8 ± 0.9	4.7 ± 1.3
5	CL (mean, %)	5.5 ± 0.6	2.6 ± 1.1	7.1 ± 0.4
6	PE (mean, %)	89.5 ± 1.2	90.1 ± 1.8	87.3 ± 0.2

[a] Cultures were grown for 2 h, treated with the drug, and incubated for 4 h before harvesting.
[b] Data for cultures grown for 6 h with 0% methanol showed no difference to that grown with 1% (data not shown).

A comparison of the lipid profiles of the 2 h control and 2 h D period (elongated) cultures indicates that the PG fraction is larger (factor of ~1.7) in D period cells than in cultures of which most of the cells are in the B period (represented by the control/unsynchronised cultures). By contrast, the relative size of the CL fraction is about 50% smaller when cells are at their most elongated, with respect to ordinary cell populations. As cell elongation increases cell size by around a factor of two, this suggests that the CL fraction is static during this period of the cell cycle. The same observations were also made in cell cultures of MG1655 stopped in the D period after 6 h growing time (Supplementary Fig. 2). The results also suggest that PA, the progenitor of all lipids in *E. coli*, follows the same trend through the cell cycle as CL. However, the size of the PA fraction is less than 1% (Table 1), making this less clear.

Despite there being no measurable growth of the CL fraction during cell elongation, the lipid profile of control cultures as a function of culture age (Supplementary Fig. 2) indicates that the absolute amount of CL must grow during culture growth, otherwise its concentration would fall as cells divided and cultures aged. The data from this study therefore suggest that the CL fraction only grows when the cells are *not* elongating. We can find no external factor that would inhibit or promote CL synthase (Cls) activity under the conditions used in this study. For example, inhibition

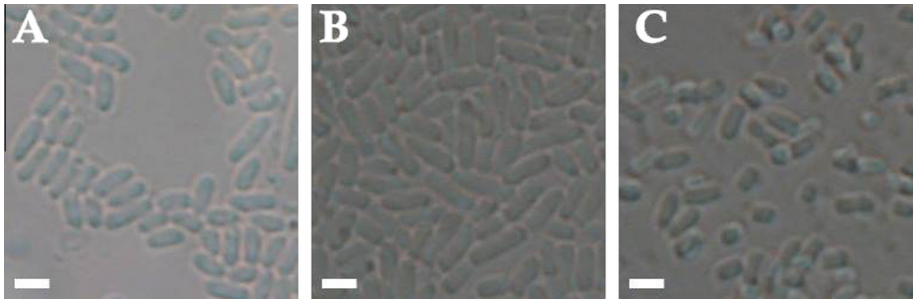

Fig. 1. Microscopy (100× magnification) of cultures of *E. coli* MG1655: (A) 2 h incubation; (B) 2 h incubation, treated with rifampicin, incubated for 4 h more before harvesting; (C) 6 h incubation. Bar = 2 μm.

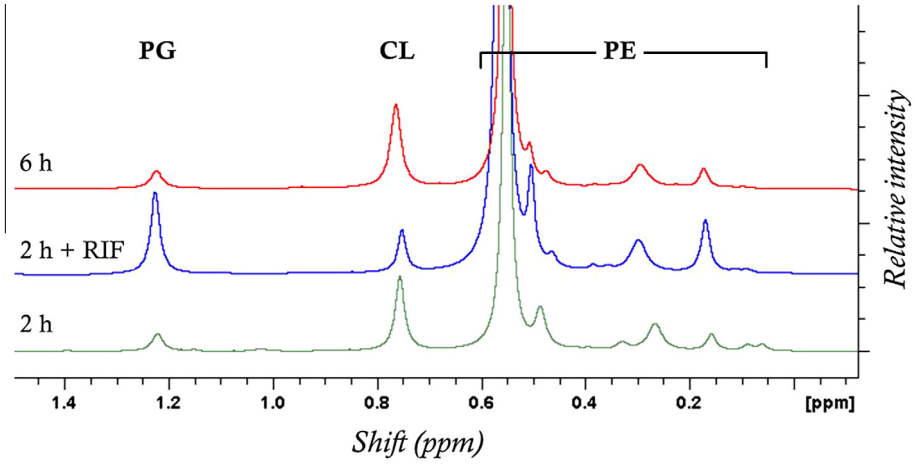

Fig. 2. Example solution-phase ^{31}P NMR spectra of lipidomes from cultures at different growth stages (2 h and 6 h) and at the D period (2 h + RIF). PG proportions are similar at 2 and 6 h. The PA resonances have a chemical shift of 4.8–5.2 ppm, this region is not shown for clarity. There are several resonances for PE due to the presence of trace sodium and potassium cations and interaction of these adducts with the solvents [38]. RIF = rifampicin.

of Cls during rifampicin-induced synchronisation, would need to abolish the activity of the three different synthases [45] completely, instantaneously and for several hours, in order to give rise to the results above. Furthermore, there is currently no published evidence of rifampicin as a promotor or an inhibitor of any lipid synthase. Moreover, there is not yet evidence of an endogenous CL degradation pathway and so there is no obvious way that degradation of CL could be induced or increased under these conditions. It is therefore appealing to speculate that CL is produced during the formation of the septum, in preparation of the new poles, and therefore that this production is a function of the cell cycle.

4. Discussion and conclusions

In this paper, we have presented evidence that *E. coli* MG1655 modulates its phospholipid profile through the cell cycle. These data indicate that the PE fraction remains dominant and does not change appreciably during the elongation in the D period. The PG fraction grows rapidly during the D-period cell elongation, whilst the CL fraction does not grow at all during this stage. The latter result is consistent with the CL fraction being located in a part of the membrane that does not grow during elongation, adding to the growing body of evidence that shows that CL is situated at the poles [23,24]. These results also suggest that production of PG, the principal precursor of CL, may occur near the septa of the cell. Our data therefore indicate that lipid production is regulated in a manner that either determines the cell cycle or is dependent on it. However, it is not clear what the control mechanism is. The most likely explanation at present is that it involves changes in the location and/or activity of lipid synthase enzymes. However, although the locus of membrane proteins in *E. coli* is beginning to emerge [46,47], the distribution of lipid synthase enzymes in the membrane is not yet fully understood.

The evidence from this study and those that show inhomogeneous lipid distribution in *E. coli* are in apparent contrast with knock-out studies of *E. coli* strains that show that no single lipid is indispensable. For example, it has been shown that neither CL [45,48] nor PE [14] is essential and even that *E. coli* without both PG and CL can grow normally below 40 °C [49,50]. These changes do not happen in isolation, however: strains without the anionic lipid CL have a higher PG fraction [45,48], and the PA fraction of strains without PG or CL is considerably higher than in strains that have not had the PG synthase genes knocked out [49,50]. It may therefore be argued that one anionic lipid is being replaced with another. Cultures without PE are viable only when there is CL and divalent cations [14] or the negative (type II) curvature-inducing lipid monoglucosyldiglyceride (MGDG) [51] to replace it, suggesting an absolute requirement for type II lipids. This suggests that where a principal lipid is not present, the culture can remain viable if another lipid with similar physico-chemical properties replaces it. This in turn suggests that the role of the different types of phospholipids in the cell envelope of *E. coli* is primarily biophysical.

The implication of a biophysics-based interpretation of the role of phospholipids in *E. coli*, that changes as a function of the cell cycle, is that different biophysical properties are required at different points in the cycle. This interpretation is consistent with evidence that the spatial distribution of lipids in the cell envelope is inhomogeneous, *e.g.* formation of the 'Z-ring' [18] and the division plane [19], and for DNA replication [20–22]. The results from this study suggest that it is desirable for the biophysical properties to be similarly inhomogeneous during different periods of the cell cycle.

It may be possible to test the hypothesis that different biophysical properties are optimum at different points of the cell cycle using synthetic cells (protocells). Current research on synthetic cells suggests that control of cell division can be determined either by replication of nucleic acids, or the membrane components. However, the two must be coordinated in order that neither one out-produces the other [52]. Finally, the data from this study are broadly consistent with data from lipid profiling of eukaryotic cells that shows the profile of HeLa cells is dependent upon the cell cycle [53,54], and early indications from genetic analysis of lokiarchaeota that show considerable membrane remodelling capabilities [55]. Taken together, these studies lend support to the hypothesis that control of the production of membrane components such as lipids is required for successful cell division in all living organisms.

Acknowledgments

S.F. would like to thank Drs. J. Löwe, S. Liddell and T. Allers and Mr. D. Mesquita da Fonseca for helpful discussions, Frans Kindt and Ronald Leito for assistance with microscopy, and the research programme of the bio-based ecologically balanced sustainable industrial chemistry (BE-BASIC) project for funding. The Dutch organisation for scientific research De Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) is acknowledged for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.07.043.

References

[1] Haeusser, D.P. and Levin, P.A. (2008) The great divide: coordinating cell cycle events during bacterial growth and division. Curr. Opin. Microbiol. 11, 94–99.
[2] Boye, E., Stokke, T., Kleckner, N. and Skarstad, K. (1996) Coordinating DNA replication initiation with cell growth: differential roles for DnaA and SeqA proteins. Proc. Natl. Acad. Sci. 93, 12206–12211.
[3] Ferullo, D.J. and Lovett, S.T. (2008) The stringent response and cell cycle arrest in *Escherichia coli*. PLoS Genet. 4, e1000300.
[4] Bates, D., Epstein, J., Boye, E., Fahrner, K., Berg, H. and Kleckner, N. (2005) The *Escherichia coli* baby cell column: a novel cell synchronization method provides new insight into the bacterial cell cycle. Mol. Microbiol. 57, 380–391.
[5] Dinarina, A., Perez, L.H., Davila, A., Schwab, M., Hunt, T. and Nebreda, A.R. (2005) Characterization of a new family of cyclin-dependent kinase activators. Biochem. J. 386, 349–355.
[6] Dorée, M. and Hunt, T. (2002) From Cdc2 to Cdk1: when did the cell cycle kinase join its cyclin partner? J. Cell Sci. 115, 2461–2464.
[7] Evans, T., Rosenthal, E.T., Youngblom, J., Distel, D. and Hunt, T. (1983) Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell 33, 389–396.
[8] Margolin, W. and Bernander, R. (2004) How do prokaryotic cells cycle? Curr. Biol. 14, R768–R770.
[9] Arends, S.J.R. and Weiss, D.S. (2004) Inhibiting cell division in *Escherichia coli* has little if any effect on gene expression. J. Bacteriol. 186, 880–884.
[10] Rueda, S., Vicente, M. and Mingorance, J. (2003) Concentration and assembly of the division ring proteins FtsZ, FtsA, and ZipA during the *Escherichia coli* cell cycle. J. Bacteriol. 185, 3344–3351.
[11] Si, F., Busiek, K., Margolin, W. and Sun, Sean X. (2013) Organization of FtsZ filaments in the bacterial division ring measured from polarized fluorescence microscopy. Biophys. J. 105, 1976–1986.
[12] Seddon, J.M. and Templer, R.H. (1995) (Lipowsky, R. and Sackman, E., Eds.), The Handbook of Biological Physics, vol. I, Elsevier Science.
[13] Cullis, P.R. and De Kruijff, B. (1979) Lipid polymorphism and the functional roles of lipids in biological membranes. Biochim. Biophys. Acta 559, 399–420.
[14] Killian, J.A., Koorengevel, M.C., Bouwstra, J.A., Gooris, G., Dowhan, W. and de Kruijff, B. (1994) Effect of divalent cations on lipid organization of cardiolipin isolated from *Escherichia coli* strain AH930. Biochim. Biophys. Acta 1189, 225–232.
[15] Barriga, H.M.G., Tyler, A.I.I., McCarthy, N.L.C., Parsons, E.S., Ces, O., Law, R.V., Seddon, J.M. and Brooks, N.J. (2015) Temperature and pressure tuneable swollen bicontinuous cubic phases approaching nature's length scales. Soft Matter.
[16] Furse, S., Brooks, N.J., Tate, E.W., Seddon, A.M., Woscholski, R., Templer, R.H., Gaffney, P.R.J. and Ces, O. (2012) Lipid membrane curvature induced by distearoyl phosphatidylinositol 4-phosphate. Soft Matter 8, 3090–3093.
[17] Tyler, A.I.I., Barriga, H.M.G., Parsons, E.S., McCarthy, N.L.C., Ces, O., Law, R.V., Seddon, J.M. and Brooks, N.J. (2015) Electrostatic swelling of bicontinuous cubic lipid phases. Soft Matter.
[18] Mileykovskaya, E. and Dowhan, W. (2005) Role of membrane lipids in bacterial division-site selection. Curr. Opin. Microbiol. 8, 135–142.
[19] Renner, L.D. and Weibel, D.B. (2012) MinD and MinE interact with anionic phospholipids and regulate division plane formation in *Escherichia coli*. J. Biol. Chem. 287, 38835–38844.
[20] Sekimizu, K. and Kornberg, A. (1988) Cardiolipin activation of dnaA protein, the initiation protein of replication in *Escherichia coli*. J. Biol. Chem. 263, 7131–7135.
[21] Xia, W. and Dowhan, W. (1995) In vivo evidence for the involvement of anionic phospholipids in initiation of DNA replication in *Escherichia coli*. Proc. Natl. Acad. Sci. 92, 783–787.
[22] Newman, G. and Crooke, E. (2000) DnaA, the initiator of *Escherichia coli* CHROMOSOMAL replication, is located at the cell membrane. J. Bacteriol. 182, 2604–2610.
[23] Oliver, P.M., Crooks, J.A., Leidl, M., Yoon, E.J., Saghatelian, A. and Weibel, D.B. (2014) Localization of anionic phospholipids in *Escherichia coli* cells. J. Bacteriol. 196, 3386–3398.
[24] Renner, L.D. and Weibel, D.B. (2011) Cardiolipin microdomains localize to negatively curved regions of *Escherichia coli* membranes. Proc. Natl. Acad. Sci. 108, 6264–6269.
[25] Mileykovskaya, E. and Dowhan, W. (2000) Visualization of phospholipid domains in *Escherichia coli* by using the cardiolipin-specific fluorescent dye 10-N-nonyl acridine orange. J. Bacteriol. 182, 1172–1175.
[26] Ballesta, J.P.G. and Schaechter, M. (1971) Effect of shift-down and growth inhibition on phospholipid metabolism of *Escherichia coli*. J. Bacteriol. 107, 251–258.
[27] Zuchowski, C. and Pierucci, O. (1978) Phospholipid turnover during the division cycle of *Escherichia coli*. J. Bacteriol. 133, 1533–1535.
[28] Mozharov, A.D., Shchipakin, V.N., Fishov, I.L. and Evtodienko, Y.V. (1985) Changes in the composition of membrane phospholipids during the cell cycle of *Escherichia coli*. FEBS Lett. 186, 103–106.
[29] Carty, C.E. and Ingram, L.O. (1981) Lipid synthesis during the *Escherichia coli* cell cycle. J. Bacteriol. 145, 472–478.
[30] Blattner, F.R., Iii, G.P., Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Jason, G., Nelson Wayne, D., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B. and Shao, Y. (1997) The complete genome sequence of *Escherichia coli* K-12. Science 277, 1453–1469.
[31] Boye, E. and Løbner-Olesen, A. (1991) Bacterial growth control studied by flow cytometry. Res. Microbiol. 142, 131–135.
[32] Boye, E., Lobnerolesen, A. and Skarstad, K. (1988) Timing of chromosomal replication in *Escherichia coli*. Biochim. Biophys. Acta 951, 359–364.
[33] Skarstad, K., Boye, E. and Steen, H.B. (1986) Timing of initiation of chromosome-replication in individual *Escherichia coli* cells. EMBO J. 5, 1711–1717.
[34] Stokke, C., Waldminghaus, T. and Skarstad, K. (2011) Replication patterns and organization of replication forks in Vibrio cholerae. Microbiology 157, 695–708.
[35] Cremonini, M.A., Laghi, L. and Placucci, G. (2004) Investigation of commercial lecithin by P-31 NMR in a ternary CUBO solvent. J. Sci. Food Agric. 84, 786–790.
[36] Culeddu, N., Bosco, M., Toffanin, R. and Pollesello, P. (1998) P-31 NMR analysis of phospholipids in crude extracts from different sources: improved efficiency of the solvent system. Magn. Reson. Chem. 36, 907–912.
[37] Furse, S., Liddell, S., Ortori, C.A., Williams, H., Neylon, D.C., Scott, D.J., Barrett, D. A. and Gray, D.A. (2013) The lipidome and proteome of oil bodies from *Helianthus annuus* (common sunflower). J. Chem. Biol. 6, 63–76.
[38] Murgia, S., Mele, S. and Monduzzi, M. (2003) Quantitative characterization of phospholipids in milk fat via P-31 NMR using a monophasic solvent mixture. Lipids 38, 585–591.
[39] Klug, R.M. and Benning, C. (2001) Two enzymes of diacylglyceryl-O-4′-(N, N, N,-trimethyl)homoserine biosynthesis are encoded by btaA and btaB in the purple bacterium *Rhodobacter sphaeroides*. Proc. Natl. Acad. Sci. 98, 5910–5915.
[40] Riekhof, W.R., Andre, C. and Benning, C. (2005) Two enzymes, BtaA and BtaB, are sufficient for betaine lipid biosynthesis in bacteria. Arch. Biochem. Biophys. 441, 96–105.
[41] Furse, S. and Killian, J.A. (2013) Lipase activity in lipidomics – a hidden problem? Mol. Membr. Biol. 30, 347–349.
[42] Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Canad. J. Biochem. Phys. 37, 911–917.
[43] Furse, S., Egmond, M.R. and Killian, J.A. (2014) Isolation of lipids from biological samples. Mol. Membr. Biol., http://dx.doi.org/10.3109/09687688.2015.1050468.
[44] Raetz, C.R. (1978) Enzymology, genetics, and regulation of membrane phospholipid synthesis in *Escherichia coli*. Microbiol. Rev. 42, 614–659.
[45] Tan, B.K., Bogdanov, M., Zhao, J., Dowhan, W., Raetz, C.R.H. and Guan, Z. (2012) Discovery of a cardiolipin synthase utilizing phosphatidylethanolamine and phosphatidylglycerol as substrates. Proc. Natl. Acad. Sci. 109, 16504–16509.
[46] Romantsov, T., Battle, A.R., Hendel, J.L., Martinac, B. and Wood, J.M. (2009) Protein localization in *Escherichia coli* cells: comparison of the cytoplasmic membrane proteins ProP, LacY, ProW, AqpZ, MscS, and MscL. J. Bacteriol. 192, 912–924.
[47] Kitagawa, M., Ara, T., Arifuzzaman, M., Ioka-Nakamichi, T., Inamoto, E., Toyonaga, H. and Mori, H. (2005) Complete set of ORF clones of *Escherichia coli* ASKA library (A Complete Set of *E. coli* K-12 ORF Archive): unique resources for biological research. DNA Res. 12, 291–299.
[48] Shibuya, I., Miyazaki, C. and Ohta, A. (1985) Alteration of phospholipid composition by combined defects in phosphatidylserine and cardiolipin synthases and physiological consequences in *Escherichia coli*. J. Bacteriol. 161, 1086–1092.
[49] Kikuchi, S., Shibuya, I. and Matsumoto, K. (2000) Viability of an *Escherichia coli* pgsANull mutant lacking detectable phosphatidylglycerol and cardiolipin. J. Bacteriol. 182, 371–376.
[50] Matsumoto, K. (2001) Dispensable nature of phosphatidylglycerol in *Escherichia coli*: dual roles of anionic phospholipids. Mol. Microbiol. 39, 1427–1433.
[51] Wikström, M., Xie, J., Bogdanov, M., Mileykovskaya, E., Heacock, P., Wieslander, Å. and Dowhan, W. (2004) Monoglucosyldiacylglycerol, a Foreign lipid, can substitute for phosphatidylethanolamine in essential membrane-associated functions in *Escherichia coli*. J. Biol. Chem. 279, 10484–10493.
[52] Blain, J.C. and Szostak, J.W. (2014) Progress towards synthetic cells. Annu. Rev. Biochem. 83, 615–640.
[53] Atilla-Gokcumen, G.E., Muro, E., Relat-Goberna, J., Sasse, S., Bedigian, A., Coughlin, Margaret L., Garcia-Manyes, S. and Eggert, Ulrike S. (2014) Dividing cells regulate their lipid composition and localization. Cell 156.
[54] Hague, C.V., Postle, A.D., Attard, G.S. and Dymond, M.K. (2013) Cell cycle dependent changes in membrane stored curvature elastic energy: evidence from lipidomic studies. Faraday Discuss. 161, 481–497.
[55] Spang, A., Saw, J.H., Jorgensen, S.L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A.E., van Eijk, R., Schleper, C., Guy, L. and Ettema, T.J.G. (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521, 173–179.