Hypothesis

A hypothesis to explain division site selection in *Escherichia coli* by combining nucleoid occlusion and Min

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Abstract The positioning of the site of cell division in *Escherichia coli* results, it is generally believed, from the operation of nucleoid occlusion in combination with the Min system. Nucleoid occlusion prevents division over the nucleoids and directs it by default to the mid-cell region between segregating nucleoids or to polar regions while the Min system prevents division in polar regions. Unresolved questions include how these systems interact to control the earliest known event in division, the assembly at the membrane of the tubulin-like protein, FtsZ, and, more importantly, what exactly constitutes a division site. Evidence exists that (1) the coupled transcription, translation and insertion of proteins into membrane (transertion), can structure the cytoplasmic membrane into phospholipid domains, (2) the MinD protein can convert vesicles into tubes and (3) a variety of membranous structures can be observed at mid-cell. These data support a model in which transertion from the segregating daughter chromosomes leads to the formation of a distinct proteolipid domain between them at mid-cell; the composition of this domain allows phospholipid tubes to extend like fingers into the cytoplasm; these tubes then become the substrate for the dynamic assembly and disassembly of FtsZ which converts them into the invaginating fold responsible for division; the Min system inhibits division at unwanted sites and times by removing these tubes especially at the cell poles.

Key words: Membrane domain; FtsZ; Min; Bacterium; Cell division

1. Introduction

At least five steps in the division of bacteria such as *Escherichia coli* can be discerned: (1) formation of the equatorial division site and recruitment of the tubulin-like protein FtsZ to it, (2) elimination of competing sites, (3) invagination of the cytoplasmic membrane (accompanied by peptidoglycan), (4) fusion of the bilayers, and (5) inactivation of the division site. The first of these steps, commonly acknowledged to be the earliest, is the formation of a polymeric, ring-like structure of FtsZ between the daughter chromosomes [1]. In the model bacterium, *E. coli*, two mechanisms currently vie to explain the positioning of this ring. The first mechanism is termed nucleoid occlusion referring to the apparent emission of an inhibitory signal by the nucleoid which generally prevents division occurring over it but permits division between segregated nucleoids [2,3] but see [4]; this would prevent the guillotining of the nucleoid by septation except in mutants such as *mukB*, *ftsK*, *dif*, and *xerCD* [5,6]. The nucleoid occlusion model has been combined with ideas about the emission of a positive signal at termination of replication that would trigger division in *E. coli* [7] to give an integrated model for the timing and positioning of division from which only the molecular details are absent (although it should be noted that termination itself does not play this role in *Bacillus subtilis* or *Caulobacter crescentus*). The second mechanism for positioning the ring, the Min system, owes its name to the aberrant division near poles that in certain mutants of *E. coli* produces a minicell and a filament [8,9]. The current paradigm is that MinC inhibits Z-ring assembly in areas determined by its interaction with MinD whilst MinE stimulates the relocalisation of MinD from membrane to cytoplasm [10,11]. In *E. coli*, the Min proteins oscillate between extended polar regions [12–14]. These oscillations have been modelled using differential diffusion rates to show how the division inhibitor MinC would be lower in the cell centre so allowing formation of the Z-ring there [15–17]. Min oscillation is not however the key to division site selection in all bacteria since it is not observed in, for example, *B. subtilis* [18] where MinCD is concentrated in the polar regions by interaction with the pole-anchored DivIVA [19]; indeed, it has been suggested that a physical property of the poles is responsible for attracting DivIVA [20] and that the function of the Min system is to prevent polar divisions [21]. The situation has been further complicated by the discovery that the Min proteins are organised together into extended membrane-associated structures that coil around the cytoplasm between the two poles [22].

Both nucleoid occlusion and the Min system can operate independently. In Min mutants, division still occurs but to either side of the nucleoids as well as between them – so nucleoid occlusion is still operating [23]. In the anucleate cells produced in other mutants, the FtsZ ring is still positioned centrally but much less precisely than when the nucleoids are present [24]. Fundamental questions still to be resolved include the molecular nature of the nucleoid occlusion mecha-

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nism and its coupling, if any, to the Min system. Changes in the structure of membranes are intrinsic to division and one evident candidate for the occlusion mechanism and for the coupling is the membrane itself. Here we propose that in bacteria (1) cell division requires the conversion of phospholipid tubes into an invagination fold by FtsZ at a membrane domain between the chromosomes and (2) the two mechanisms for division site selection are combined in the competition for phospholipid structures between FtsZ and Min.

2. The model

Our membrane model is in three parts:

1. Coupled transcription–translation–insertion of proteins into and through membrane – *transertion* – and the consequent crowding of active ribosome–translocase complexes around the chromosomes create heterogeneity in the plane of the membrane. Proteolipid domains with distinct physical-chemical characteristics form around the daughter chromosomes and a very different proteolipid domain forms between them. This heterogeneity provides the basis for nucleoid occlusion: the domains around the chromosomes do not favour the formation of tubes and the polymerisation of FtsZ whilst the domain between them does (Figs. 1A and 2A).
2. This division domain is enriched in lipids such as cardiolipin and short chain phospholipids but is short of bilayer-stabilising proteins. This domain increases in size as bacterial growth and chromosome segregation continue. The composition of the division domain favours the transition from a membrane that is essentially flat to one in which a variety of structures forms. These structures include dimples, buds and tubes that extend into the cytoplasm [25] and, as the domain grows, the frequency of formation of these structures increases (Fig. 1B). These tubes (and similar structures such as folds) are the substrate of FtsZ. Binding of enough FtsZ to enough of these tubes generates the FtsZ polymer that executes division by orchestrating phospholipid structures into an invagination fold. There are many ways in which this might happen. For example, an FtsZ protofilament binding to the base of a phospholipid tube or even just a dimple might nucleate the assembly of protofilaments into a larger sheet comprising several FtsZ protofilaments. This change would be accompanied by a conformational change from curvature of the single protofilament in the plane of a flat membrane to the curvature of the sheet perpendicular to the membrane; such conformational switching would then stretch the phospholipid tube into a fold (Fig. 2B). An alternative mechanism to drive invagination via the release of mechanically stored energy could be based on *disassembly* of a curved sheet or, in the limit, a microtubule as proposed for tubulin [26] (Fig. 2C). Finally, the mechanisms proposed above could operate in more than one place at the same time using separate protofilaments. Fig. 2D shows what happens when two protofilaments start to form near one another (there may be more protofilaments than this): for example, the simultaneous formation of separate assemblies of protofilaments in different parts of the interface of a roughly circular domain stretches this domain along one axis using the mechanism of Fig. 2B (the mechanism of Fig. 2C could also have been invoked).

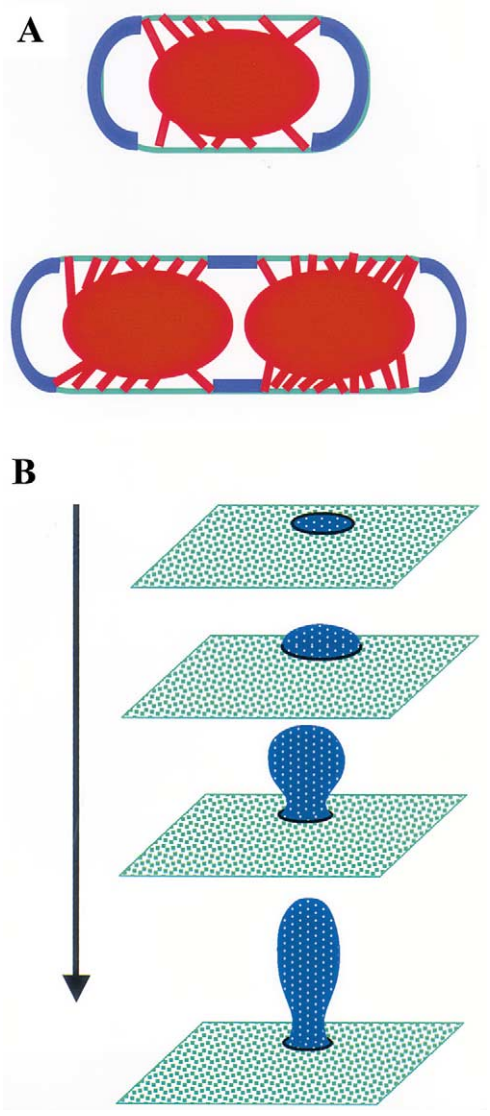

Fig. 1. A: Domains of specific phospholipid composition and structure form at the poles and in between the chromosomes (blue lines) whilst different domains (green lines) form as a result of transertion (red bars) around chromosomes (red ovals). B: An increasing quantity of cardiolipin or short chain phospholipids (blue) in a flat sheet of other phospholipids (green) forms first a domain and then a dimple, a bud and a tube. The boundary between the two types of lipids is shown by the black line. The arrow indicates the increase in cardiolipin and accompanying changes in the structures although a dynamic equilibrium between these structures may exist. (For interpretation of the references to colour in the figure legends, the reader is referred to the web version of this article.)

3. Cell division leaves some of these lipids at the poles where they may again give rise to tubes. Tubes may also form occasionally in other regions outside the central division domain. Such tubes may initiate FtsZ assembly, and hence division, in the wrong place. A mechanism is therefore required to eliminate tubes forming outside the central division domain. This is the task of the Min system. Min achieves this by binding to the same substrate as FtsZ namely a phospholipid tube which turns into a vesicle thereby destroying the division marker (Fig. 3). (Note (i) the binding of MinC to MinD probably increases the affinity of the MinC/MinD complex for its target [27] and (ii)

the MinCD complex probably abrogates FtsZ polymerisation directly [28]; these can be considered in our model as complementary albeit important mechanisms.) Preferential action of Min away from the division domain (e.g. at the poles) is achieved by some MinD being left behind after its action to act as a nucleation site at the poles (Fig. 4). This residual, polar, MinD is then extended into polymers along the membrane; finally, MinE binding to these polymers stimulates their depolymerisation (Fig. 4). The segregation of the chromosomes creates a new central domain producing phospholipid tubes for which FtsZ can compete effectively with MinD (Fig. 4B).

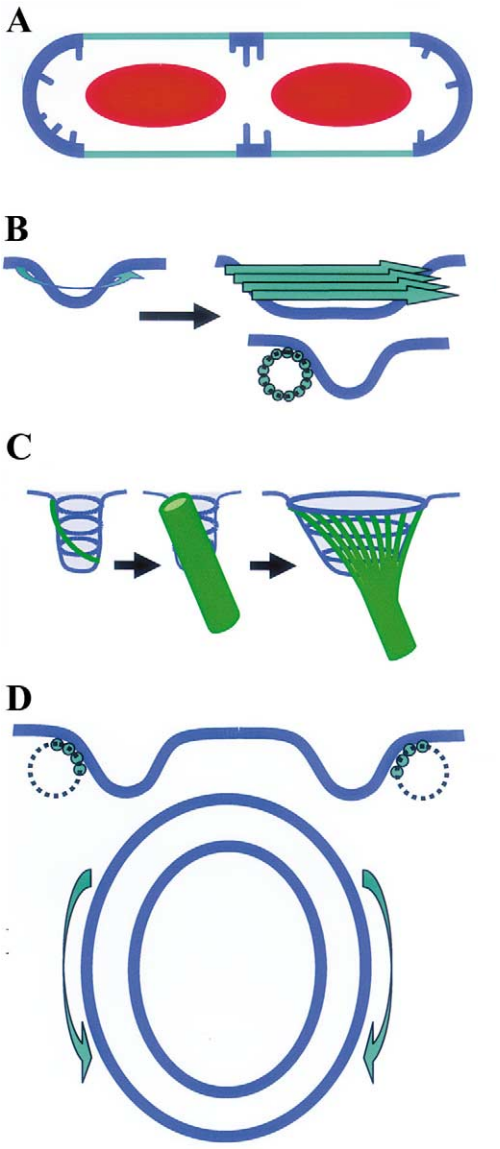

Fig. 2. A: Domains of specific phospholipid composition (blue and green) in the plane of the membrane lead to the formation of phospholipid tubes (blue) between the segregated chromosomes (red ovals). B: Protofilament of FtsZ (green curved arrow) polymerises on a phospholipid tube and nucleates the formation of a sheet of several parallel protofilaments of FtsZ that is curved in a different dimension. This conformational switching converts the phospholipid tube (shown here as a dimple) into an invagination fold. A cross-section shows the curvature of the sheet of the parallel protofilaments. C: Protofilament of FtsZ (green spiral) polymerises on a phospholipid tube and nucleates the formation of a larger structure (such as a microtubule) containing several protofilaments of FtsZ (green). Conformational switching and disassembly of the large FtsZ structure into a sheet of filaments converts the phospholipid tube into an invagination fold. D: Formation of two curved sheets of protofilaments (as in the mechanism in B) in opposite sectors of a large circular domain stretches it into a long ellipse.

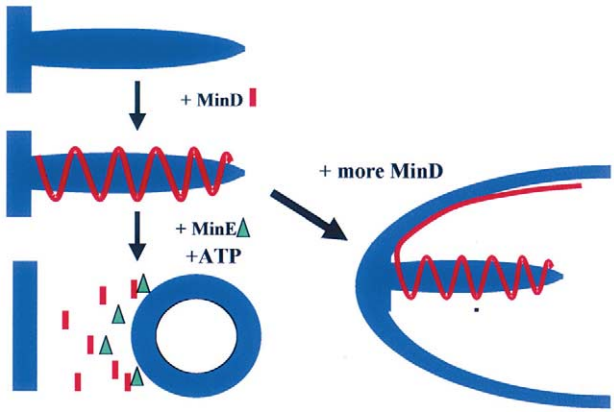

Fig. 3. MinD (red blocks) binds to a phospholipid tube projecting from the membrane (blue) and polymerises (red spiral). Addition of more MinD results in this polymer extending along the membrane towards the equator. Alternatively, addition of MinE (green triangle) and ATP releases both MinD and the phospholipid tube to generate a vesicle (blue circle).

3. The model allows the following questions to be answered

(1) Why should Min act preferentially at poles?
(2) Why should Min oscillate in some bacteria?
(3) How can the range of mutant phenotypes be generated?

3.1. Why should Min act preferentially at poles?

Consider a newborn cell. The poles are the regions where tubes form most readily since a new division domain has yet to form. MinD is therefore localised at the poles. As a tube forms, MinD competes with FtsZ to bind to it (note that in the absence of MinD, FtsZ acts on tubes either at the poles to form a minicell or at the equator). MinE may have the function of cutting the tube away from the membrane (like a surgeon shearing a polyp from the intestine) to generate a vesicle and the function of releasing the other proteins from the vesicle (Fig. 3). Continued tube formation in a pole retains MinD in that pole as long as (1) the rate of tube formation in that pole is high; this rate itself is a function of the rate of formation of tube-forming proteolipid domains as mediated by transertion (and hence is related to the ratio of synthesis of tube-forming phospholipids and of the rate of synthesis of proteins that oppose such tube formation), (2) MinD is sufficiently abundant, (3) MinD has an affinity for itself and (4) the vesicles are large, i.e. if the shearing rate (due to MinE concentrations or activity) is low such that the tubes and associated MinD have the chance to grow. In addition, the shearing event may leave enough MinD behind in the membrane of the pole for it to nucleate the binding of other MinD proteins to form polymers that therefore have one end in this pole; if these MinD polymers are unstable and depolymerise from the other end (i.e. towards the poles) this will again lead to the concentration of MinD being highest in the poles so allowing the central domain to develop and initiate division. For example, a probability of binding of MinE to MinD poly-

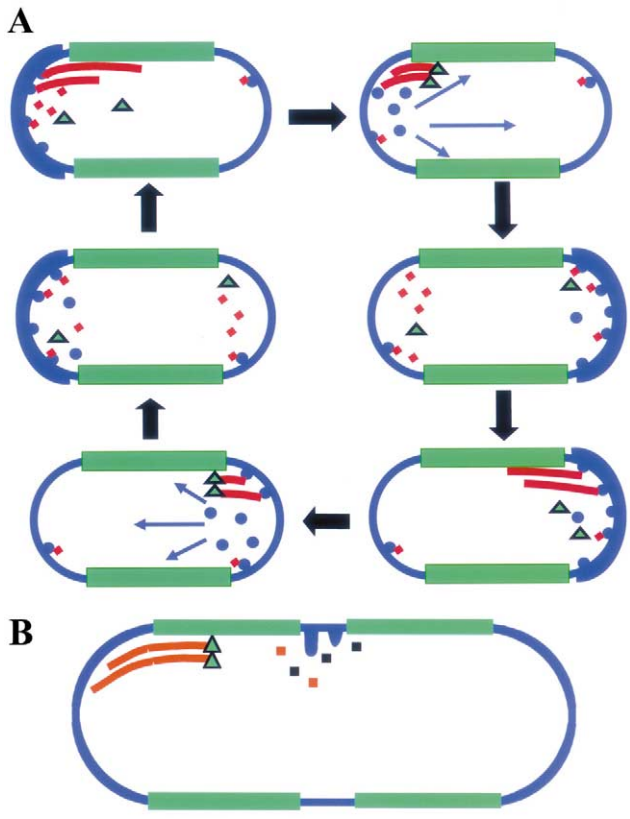

Fig. 4. A: Min oscillation in an *E. coli* cell in which the chromosomes have not segregated. MinD polymers (red lines) start at one pole and depolymerise when MinE (green triangle) binds (top right of figure). Tube-forming phospholipids (blue) are at poles whilst non-tube-forming phospholipids (green) are in domain constructed by centrally placed chromosome (see Fig. 1A). Cooperative action of MinE leads to synchronous depolymerisation. Relative proportions of tube-forming phospholipids (blue rectangles) in the two poles change as a result of Min action being concentrated on one pole at a time and the blue arrows indicate the direction of relative enrichment of these phospholipids as a result of excision from the pole and of diffusion through the membrane and cytoplasm to the other pole (plus new synthesis). Eventually, there are sufficient of these lipids (thick blue line) to generate tubes and hence to retain MinD at the other pole and to nucleate polymers from it. Some MinD remains bound to lipid in each pole throughout the cycle. The temporal sequence lasts about a minute. B: Competition between MinD (red rectangles) and FtsZ (black rectangles) for binding to phospholipid tubes at the domain in the centre created by the segregating chromosomes.

mer that was proportional to polymer length would bias the length distribution to short lengths and so tend to retain MinD in polar regions; a preferential binding of MinE to the distal end of the polymer would increase this bias (Fig. 4).

3.2. Why should Min oscillate in some bacteria?

Although Min oscillation is not crucial to division site selection in our model, it is easy to see how it can arise since MinE displaces MinD as in other reaction–diffusion models [15,16]. The situation is, however, more complicated in our model because an important role is played by firstly the availability of the substrate, the phospholipid tubes, which bind MinD and which nucleate MinD polymers and secondly the polymerisation and depolymerisation of MinD from the poles. If, after shearing off a tube from a pole, there are tubes at the other pole then the MinD liberated from the vesicle can diffuse to the other pole provided a tube has formed there (and provided that few tubes and MinD remain in the first pole). Hence, MinD localisation and oscillation is partly under the control of polar membrane domains. These domains also facilitate oscillation by providing a starting point for polymerisation whilst synchronised depolymerisation could result from a cooperative process, for example, a long polymer being depolymerised by MinE could meet a shorter polymer and trigger its depolymerisation too (Fig. 4). Note that in filaments in which MinD oscillates between segregated nucleoids, our explanation is that unused division domains between the nucleoids generate the tubes that are the substrate for Min; Min then oscillates between these domains. It should also be noted that there may be oscillations in the relative proportions of phospholipids in the poles (Fig. 4). One possibility is that key tube-forming lipids only go from one pole to a vesicle that does not diffuse far and then back to the same pole. Another possibility is these lipids do diffuse from one pole to the other via a variety of mechanisms (distinguishing between these possibilities will require information on the lifetimes of the vesicles, i.e. fission/fusion times, relative to their diffusion times and other factors like the capture of tube-forming lipids by nascent tubes).

3.3. How can the range of mutant phenotypes be generated?

Excess MinCD blocks division everywhere because it binds to phospholipid tubes everywhere. MinE mutants undergo division inhibition because there is no recycling of proteins bound to tubes (perhaps no recycling of the lipids in the tubes either). In MinE mutants, moreover, MinD–green fluorescent protein (GFP) appears to be distributed over the entire membrane because the tubes coated with MinD are not disassembled and form a network. MinCD mutants allow polar divisions because the tubes at the poles are used.

4. Evidence consistent with the model

4.1. Transertion structures the cytoplasmic membrane

The crowding of active ribosome–translocase complexes around the chromosomes may structure the membrane either physically by constraining the insertion of nascent proteins or chemically by selecting for those phospholipids that interact with these proteins (for evidence for the coupling of translation to insertion see [29], for other references see [30,31]). This localisation of transertion is proposed to be responsible for the existence of a distinct domain in the membrane between segregated chromosomes (Fig. 1); this domain may actually exist before the end of chromosome replication if the terminus region is in the centre of the cell since this region is relatively low in transertion activity [32]. Disruptions of transertion are predicted to disrupt domain structures and therefore alter the viscosity of membranes; such alterations in membrane dynamics are indeed observed when transcription and translation are inhibited [33]. In both *E. coli* and *B. subtilis*, membrane viscosity decreased after inhibition of protein synthesis by chloramphenicol or puromycin or inhibition of initiation of RNA synthesis by rifampicin which should destroy transertion structures and therefore disrupt membrane domains; membrane viscosity did not decrease after inhibition of RNA elongation by streptolydigin which should freeze transertion structures and therefore preserve membrane domains [33].

4.2. Domain structures are associated with division

Simultaneous observation in living *E. coli* of the nucleoids and the physicochemical state of the membrane and nucleoids via 4,6-diamino-2-phenylindole (DAPI) and FM4–64, respectively, revealed distinct fluorescent regions of different intensities around the nucleoids, between them and at the poles [34]. These results are consistent with formation of a domain between the nucleoids being an early event in division. Moreover, these results also reveal physical changes in the membrane in the 1/4 and 3/4 positions in rapidly growing cells, consistent with these changes being occasioned by the earliest stages in the partitioning of the nucleoids. Staining with the cardiolipin-specific dye 10-*N*-nonyl-acridine orange indicated the presence of large domains enriched in this phospholipid at the division site and at the poles [35]. These latter results are corroborated by the enrichment in cardiolipin found in minicells which can be considered as either isolated poles or isolated septa [36]. Conceivably, such cardiolipin-rich domains might facilitate tube formation, particularly if the concentration of calcium is locally high since calcium plus cardiolipin makes an 'inverted cone' leading to membrane curvature (for references see [37] and for a full analysis see [38]), in addition to alterations in lipid structures [39], calcium can promote lateral segregation [40] and lateral segregation of phospholipids may in turn suffice to generate endocytosis as shown in giant liposomes [41]. It should be stressed that the essential role of anionic phospholipids in *E. coli* is not specific to a particular phospholipid since the anionic phospholipids can substitute for one another [42].

4.3. Lipid vesicles are seen in some bacteria

During mild osmotic up-shocks, the relative incompressibility of the cytoplasmic membrane leads to the formation either of plasmolysis spaces or of endocytotic or exocytotic vesicles [43]. Deformations of the membrane can also form in steady state conditions and extensive systems of membrane vesicles have been described in bacteria such as *Azotobacter vinelandii* [44] and in *Mycobacterium lepraemurium* [45]. Although there have been few reports of transient structures of tubes and vesicles in *E. coli* or *B. subtilis* being observed by standard techniques of electron microscopy, absence of evidence is not necessarily evidence of absence. Indeed, giant cells obtained by ultraviolet (UV) irradiation of a *lon envB* (*mon*) double mutant of *E. coli*, show intracellular membrane vesicles and cisternae [46] whilst, more recently, vesicles enriched in cardiolipin have been produced at the poles of *E. coli* by overproduction of a peripheral membrane protein, MurG [47]. At one time, it was considered that a membranous structure, the mesosome, existed and was important in division [48,49]. The mesosome has been dismissed as a preparation artefact [49–51] but it is conceivable that preparation simply amplifies and reveals a real but labile, non-equilibrium structure. This might then be the interpretation for the mesosome-like structures observed following treatment of bacteria with a variety of drugs [52–54]; such structures accompany a remarkable increase in the frequency of septation following treatment of *Lactobacillus plantarum* with the bacteriocin, Lactocin 705 [55]. The structures we invoke are more modest than what have been described as mesosomes but both may have a common origin in a domain of distinct physical and chemical properties. Indeed, vesicles are sometimes observed in wild-type *E. coli* and, although no indications for the existence of tubes have been reported in freeze-fracture preparations of wild-type *bacteria*, invaginations of the plasma membrane resembling tubes have been reported in yeast [56] where lipid domains are implicated in cell division [57].

4.4. Lipid domains can spontaneously make tubes

The formation of a lipid domain within an essentially flat membrane suffices for budding to occur provided the tension within the membrane is small [25]. Briefly, the excess free energy associated with the line (interface) between the domain and the rest of the membrane grows linearly with the length of this line; if the domain turns into a bud connected to the membrane by a thin neck, this line is drastically shortened and the line energy therefore reduced (although bending energy increases). This means that budding must occur once the domain attains linear dimensions of 80–800 nm. This mechanism has been invoked to explain how polymers of F_0F_1 adenosine triphosphatase (ATPase) could generate the cristae in mitochondria [58].

4.5. MinD creates phospholipid tubes

MinD has an affinity in vitro for anionic phospholipids [59] and, when overproduced, generates the type of membrane abnormalities that might be expected of an excess of a protein with the capacity to organise lipids or to respond to the organisation of lipids (Lutkenhaus, personal communication). Addition of a His-tagged MinD to phospholipid vesicles in the presence of ATP led to MinD binding to the vesicles, and polymerising [60]. The physical association of MinE with these MinD polymers then led to a reorganisation of their structure and disassembly [60]. In a similar study, untagged MinD assembled into a helical array and deformed the vesicles into tubes [61]. Stimulation of the MinD ATPase by addition of MinE then led to disassembly of the arrays and the release of MinD from the vesicles (cf. Fig. 3). The significance of the transformation of vesicle into tube is, we believe, that the lowest state of energy corresponds to MinD bound to a phospholipid tube; this means that, given a choice of phospholipid structures, MinD will preferentially bind to tubular rather than to other phospholipid structures. In other words, MinD should be expected to recognise tubes.

4.6. Inhibiting phospholipid synthesis or transertion affects division

Inhibition of phospholipid synthesis results in a rapid cessation of division although continued growth allows the protein to lipid ratio to increase 60%. After phospholipid synthesis resumes, cells start dividing once the protein/phospholipid ratio returns to normal [62,63]. This is unlikely to be because of a lack of membrane material to make the septum since the cells increase considerably in length; in our model, it would be because the increase in protein to lipid inhibits the formation of lipid domains associated with septum formation. Inhibition of transertion via inhibition of transcription or translation immediately stops growth so the cessation of division in these conditions is hardly surprising.

4.7. The behaviour of FtsZ and Min is perturbed in lipid-deficient mutants of E. coli

Fusions of FtsZ and the FtsZ-interacting protein, ZipA, to the GFP often formed spiral structures in the abnormally dividing cells of an *E. coli* mutant deficient in its major phos-

pholipid, phosphatidylethanolamine [64]. Fusions to GFP also reveal that the localisation and oscillation of the Min proteins are disrupted in this strain, consistent with MinD having a lipid preference [59]. Indeed, in this strain, the distributions of GFP–MinD and of cardiolipin are similar [35]; the MinD polymers are organised into compact clusters possibly due to highly charged regions of cardiolipin increasing the cooperativity of MinD assembly [59]. Anionic phospholipids create a low pH environment at the membrane surface that favours protonation of acidic amino acids and consequently a decreased repulsion of the helix. Cardiolipin domains are a natural sink for protons in the membrane [65] that could facilitate the membrane insertion of a cluster of glutamic acid residues at one end of the MinD C-terminal motif [66,67].

4.8. Dynamin replaces FtsZ in some organelles

Dynamins are large guanosine triphosphatases (GTPases) that generate force on eukaryotic membranes leading to tube formation, endocytosis and vesicle production [68] and specific phospholipids have been implicated in their action [69]. Most mitochondria do not have FtsZ but, instead, a dynamin, DNM1, is localised to their constriction sites that when mutated causes failure to pinch off mitochondria [70]. This is consistent with the idea that FtsZ, perhaps in combination with another protein, has a similar mechanism of action.

4.9. The rate of phospholipid synthesis changes during the cell cycle

Our model is consistent with alterations in phospholipid synthesis during the cell cycle insofar as changes in the surface to volume ratio would affect – and be affected by – division. Several studies using different techniques are consistent with a doubling in the rate of phospholipid synthesis during the cell cycle of *E. coli* [71,72]. In *C. crescentus*, synthesis is restricted to two discrete periods [73]. The first period of net phospholipid synthesis begins in the swarmer cell shortly after cell division and ends when DNA replication initiates whilst the second period begins when DNA replication is about two-thirds complete and ends when DNA replication terminates.

4.10. FtsZ can interact directly with phospholipids

It might be argued that all proteins that induce tube formation interact with the bilayer through an amphipathic α-helix. A clear candidate for this helix in FtsZ is missing. In vitro, however, FtsZ polymerisation is facilitated by cationic lipids [74,75] and, in a phosphatidylethanolamine monolayer system, FtsZ makes large rings up to 500 nm in diameter and linear polymers of several microns consistent with these being the FtsZ equivalent of microtubules or other ordered assemblies [76]. Hence FtsZ can interact with phospholipid structures. This does not of course preclude FtsZ interacting with other proteins that might also bind to phospholipid structures. For example, FtsZ interacts with ZipA [77] and FtsA [78].

4.11. Division involves non-equilibrium structures

In our model, phospholipid structures that are the precursors to division are constantly being assembled and disassembled into vesicles or into a division fold between the nucleoids as mediated by a dynamic FtsZ structure. Examples of known dynamic lipid structures include the Golgi apparatus in yeast [79] whilst tubulin dynamics include switching from protofilaments that are constrained to be straight in a one-dimensional microtubule to protofilaments that curve in a two-dimensional sheet with a consequent release of energy stored mechanically [26] that could drive a change in the structure of an associated membrane. It is believed that the FtsZ ring is also extremely dynamic [80,81] and, although there is no consensus about which of the wide variety of FtsZ structures observed in vitro exists in vivo [82], 'ribbons' of eight to 25 parallel protofilaments have been observed in conditions that reflect crowding in the cytoplasm [83].

5. Predictions

1. Streptolydigin, which is believed to 'freeze' transertion structures by blocking the elongation step in transcription [33], might nevertheless allow division.
2. Production of dynamin in bacteria in which FtsZ is partially defective (either in abundance or in activity) might partially suppress their division defect. The idea is that early in evolution, the molecular mechanisms underlying key events were less specific but more robust.
3. Vesicles and tubes should be observed near poles and in the centre.
4. MinD should compete in vitro with FtsZ for interaction with lipid structures.
5. An increased production of the lipids that constitute the tubes should restore division in conditions of MinCD overproduction.
6. Oscillations of the Min proteins could be generated in bacteria in which these proteins do not normally oscillate if the polar MinD–MinD interaction is weakened.

6. Discussion

Division is intimately coupled to the growth of the cell. It is easy to see why. Cells that cannot grow cannot divide indefinitely: cells that can grow can – providing growth and division are coupled. It is also easy to see how they might be coupled – indeed, it is easy to suggest several mechanisms that may all be involved (although it is less easy to evaluate their relative contributions). During growth, proteins and phospholipids are inserted into the cytoplasmic membrane and a slight increase in the ratio of membrane area to cytoplasmic volume can cause invagination (indeed, it could be argued that invagination must happen if the synthesis of surface material is proportional to the growth of cytoplasm). Moreover, phospholipids must be translocated from one monolayer to the other and a 1% imbalance in packing can also cause invagination. Segregated nucleoids may produce a specific proteolipid domain in the membrane between them that is particularly propitious for division [31]. This gives a basis for coupling growth and division. More interestingly, it gives a mechanism for initiating division and for relating the two systems for selecting the division site: nucleoid occlusion and Min. As the division domain grows and matures it produces phospholipid tubes and vesicles that increase in number until a threshold at which FtsZ binding to them initiates cell division (note that this binding must be related to macromolecular crowding in the cytoplasm [84]). Nucleoid occlusion corresponds to the perturbation of this process by the disruption of the specific phospholipid domains necessary for tube

formation, until separation of the chromosomes. At the poles and to some extent at the centre, MinD removes the phospholipid tubes that would otherwise lead to misplaced or premature division. MinD has a polar bias because such 'aberrant' tubes are most likely to form in the cardiolipin-rich polar domains which are the remnants of the former division domain. MinD and FtsZ therefore compete for the division substrate. If this is true, it leaves a new question: 'how is FtsZ implicated in the conversion of tubes, and perhaps vesicles, into the final invagination that accomplishes division?'. Answering this question may have far-reaching repercussions given that interactions between phospholipids and tubulin-like proteins are a recurrent theme in division across the phyla.

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