Deformations in the cytoplasmic membrane of *Escherichia coli* direct the synthesis of peptidoglycan

The hernia model

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ABSTRACT To explain the growth of the Gram-negative envelope and in particular how it could be strengthened where it is weakest, we propose in the hernia model that local weakening of the peptidoglycan sacculus allows turgor pressure to cause the envelope to bulge outwards in a hernia; the consequent local alteration in the radius of curvature of the cytoplasmic membrane causes local alterations in phospholipid structure and composition that determine both the synthesis and hydrolysis of peptidoglycan. This proposal is supported by evidence that phospholipid composition determines the activity of phospho-*N*-acetylmuramic acid pentapeptide translocase, UDP-*N*-acetylglucosamine:*N*-acetylmuramic acid-(pentapeptide)-*P*-*P*-bactoprenyl-*N*-acetylglucosamine transferase, bactoprenyl phosphate phosphokinase, and *N*-acetylmuramyl-L-alanine amidase. We also propose that the shape of *Escherichia coli* is maintained by contractile proteins acting at the hernia. Given the universal importance of membranes, these proposals have implications for the determination of shape in eukaryotic cells.

INTRODUCTION

In balanced growth, the Gram-negative bacterium *Escherichia coli* elongates by inserting new peptidoglycan and excising old (for review see reference 1). Despite the turnover of up to 50% of its peptidoglycan per generation (2, but see reference 1), this process is accomplished without the loss of its cylindrical shape and without lysis. Several models have been proposed to explain how the growth of the Gram-negative wall occurs (3). These models invoke autolysins to cleave the cross-linked peptidoglycan chains. The "allosteric" model requires cleavage to occur only in the vicinity of covalently cross-linked but unstretched peptidoglycan (3); the "inside-to-outside" model as applied to thick-walled Gram-positive bacteria requires the new wall to be relaxed and later, as it is pushed outwards and elongated, to be stressed and finally cleaved (4); a "patches" model applies the inside-outside strategy to small isolated patches of the wall (5); a multienzyme model offers a transpeptidase/autolysin complex that cleaves and releases one cross-linked oligopeptidoglycan chain for every two nascent chains covalently bound to the wall (3). Most of these models have been criticized by Koch, including his own (3). The basis of his criticisms is that the Gram-negative wall is thin and unrestrained autolysin action should result in lysis; to enable the autolysins to cleave only where safe requires these molecules to have novel and implausible properties. In circumventing the lysis problem by restraining autolysin action to patches, the patches model encounters the second problem of how to maintain cylindrical shape. An additional criticism, not made by Koch (3), is that a negative-feedback mechanism is missing from these models and a weakened wall has no greater chance of being strengthened than a sound one.

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To achieve wall growth without incurring lysis, we propose a radically different model in which local changes in the radius of curvature of the cytoplasmic membrane determine synthesis and cleavage and in which contractile proteins maintain cylindrical shape. The model offers negative feedback mechanisms and certain of its requirements and implications have been calculated in the Appendix.

THE HERNIA MODEL

The weakening of the peptidoglycan sacculus by, for example, autolysin cleavage leads to a local decrease in the radius of curvature (in both longitudinal and circumferential directions) of the cytoplasmic membrane as, driven by the turgor pressure in exponential and stationary phase cells, this membrane begins to bulge through into the periplasm as a hernia (Fig. 1). The curvature of this hernia imposes different packing constraints on its outer and inner monolayers. This altered structure leads to particular phospholipids congregating in this curved region via lateral diffusion: in the outer monolayer, phospholipids with large headgroups and saturated acyl chains are selected by the constraint that this monolayer be convex; conversely, in the inner monolayer small headgroups and bulky acyl chains are selected by the constraint that this monolayer be concave (Fig. 2). The phospholipid structure and composition of the hernia prevent cleavage by autolysins by either inhibiting their activity immediately or excluding them from the region. At the same time, the hernia favors the activity of the enzymes that thicken and elongate the peptidoglycan. These enzymes include those responsible for transpeptidation, transglycosylation, and precursor synthesis. Hence weakening the peptidoglycan leads to a hernia which in turn results in new peptidoglycan being laid down. Lysis is averted and the envelope is enlarged.

The peptidoglycan is attached to the cytoplasmic

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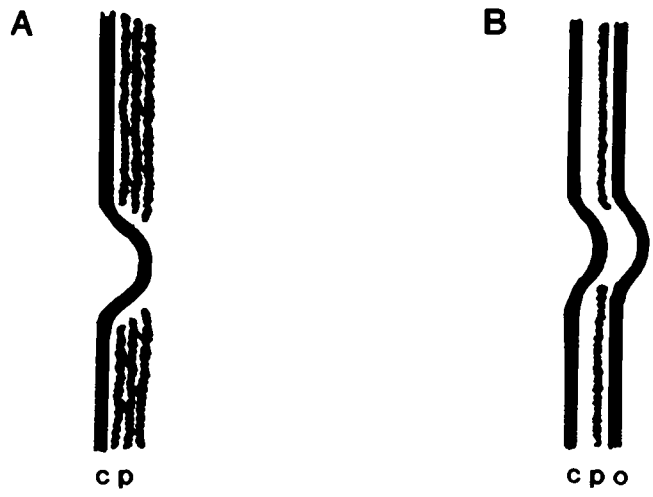


FIGURE 1 Weakened peptidoglycan results in a hernia. *c*, cytoplasmic membrane; *p*, peptidoglycan; *o*, outer membrane. (*A*) Gram-positive bacterium. (*B*) Gram-negative bacterium.

membrane by transmembrane proteins that interact with contractile proteins or that are themselves contractile. Since these proteins are also attached to a cytoskeleton, their contractions pull in the hernia (Fig. 3). As this region of the membrane becomes flatter, its radius of curvature (the radius of the circle with that curvature) increases and phospholipid composition and packing are correspondingly altered. Autolysins may now function and, projecting either outward from the cytoplasmic membrane or inward from the outer membrane, cleave the peptidoglycan closest to them. If the newly strengthened area happens to be thicker than elsewhere, autolysins will trim it and hence restore cylindrical shape. The hernia model therefore offers a homeostatic mechanism whereby weakened regions of the wall are reinforced and thickened regions are thinned.

REQUIREMENTS OF THE MODEL

1. Turgor pressure to generate the hernia at the site of the weakened wall.
2. A relationship between the curvature of membranes and phospholipid structure and composition.
3. A relationship between phospholipid structure and composition and enzyme activity.
4. A Gram-negative sacculus more than one layer thick (at least temporarily or locally).
5. Growth of the peptidoglycan sacculus in patches.
6. Interaction of contractile proteins with the peptidoglycan of the hernia.
7. Cleavage of excess oligopeptidoglycan bonds by autolysins projecting either outward from the cytoplasmic membrane or inward from the outer membrane. In this latter case the autolysins must be sensitive to the structure and/or composition of the outer membrane.

Turgor pressure

In Gram-negative bacteria turgor pressure has been calculated as ~3.5 atm. (6) while in the thicker-walled Gram-positive bacteria turgor may be as high as 20 atm. (7). These latter values have been confirmed by observing the collapse of gas vacuoles in the Gram-negative bacterium, *Ancylobacter aquaticus* (8). Such pressures have to be resisted by a material with tensile strength, in the case of *E. coli* and almost all eubacteria, peptidoglycan.

In Gram-positive bacteria in which the cytoplasmic membrane is restrained by the peptidoglycan wall, a weakening in this wall should lead to a deformation of the cytoplasmic membrane much as the inner tube of a bicycle tire may protrude through a split in the casing (Fig. 1 *a*). In Gram-negative bacteria, the peptidoglycan sacculus is located in the periplasm between the inner, cytoplasmic, membrane and the outer membrane. One study suggests that the principal source of opposition to turgor pressure is the outer membrane (6) which is attached to the peptidoglycan via the Braun lipoprotein. It is generally agreed that the periplasm can generate a

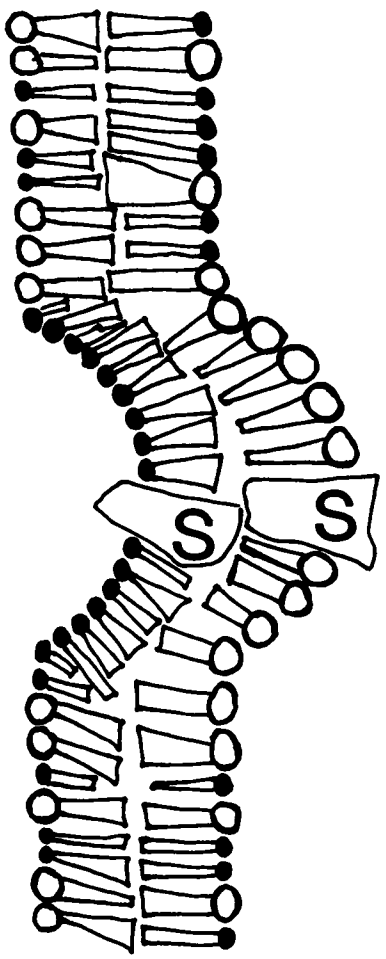


FIGURE 2 Altered monolayer composition in the hernia. *S*, enzymes synthesizing peptidoglycan.

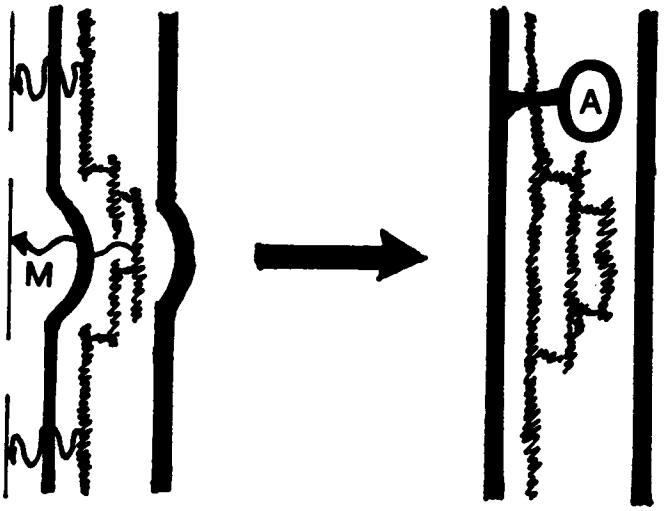


FIGURE 3 Restoration of shape by contractile proteins (*M*) and autolysins (*A*).

Donnan equilibrium across the outer membrane because of the presence of membrane-derived oligosaccharides (9). It is not agreed how much turgor pressure is opposed by the cytoplasmic membrane pushing against the peptidoglycan (10); in the extreme case, where the periplasm is isotonic with the cytoplasm, a deformation in the outer membrane would cause a corresponding deformation in the inner membrane since the distance between these membranes is only 12 nm or so (11) and the periplasm is probably an incompressible gel (12). It has indeed been suggested that the main pressure differential would be across the whole inner membrane–periplasm–outer membrane complex (10) and hence a weakening in the peptidoglycan should result in a deformation in both cytoplasmic and outer membranes (Fig. 1 *b*).

Curvature of membranes and phospholipid structure and composition

All biological membranes contain a variety of lipids that, when purified, adopt either a bilayer or a nonbilayer configuration in an aqueous environment. The molecular characteristics required for nonbilayer formation have been thoroughly studied (13–15). The configurations adopted by phospholipids depend on the "critical packing parameter" of the lipid in question (13). This parameter, $v/a_o l_c$, describes the "shape" characteristic of a particular phospholipid. In this expression, v is the volume of the fatty acid chain(s), l_c is the maximum length of the chains and a_o is the optimal surface area per amphiphile as determined by the volume of the head-group, its hydration, charge, and hydrogen-bonding capabilities. Double-chained lipids with small head-group areas such as unsaturated phosphatidylethanolamine or cardiolipin (diphosphatidylglycerol) plus calcium resemble inverted truncated cones and form inverted micelles while single-chained lipids with large head-groups resemble cones and form micelles.

The membranes of *E. coli* and its close relative *Salmonella typhimurium* contain a wide range of lipids and proteins, including phosphatidylethanolamine, phosphatidylglycerol, cardiolipin, phosphatidylserine, phosphatidic acid, and diacylglycerol; the saturated fatty acids of the phospholipids consist of lauric, myristic, palmitic, and stearic acids while the unsaturated fatty acids consist of palmitoleic and *cis*-vaccenic acids and neutral lipids; the phospolipids may also contain cyclopropane phospholipids (16–19). Generally, this heterogeneous population must adopt a bilayer structure. However, should the nonbilayer-forming species segregate laterally, an alteration of normal bilayer structure may be expected. A particular species of phospholipids has an intrinsic radius of curvature that represents the tendency of a monolayer of that species to curl in an aqueous environment (15). Hence a monolayer of a species with a small head-group and an unsaturated, bulky tail will have a relatively small radius of curvature.

The first proposed consequence of the formation of a hernia is a concomitant effect of its small radius of curvature of ~10 nm or less (see Appendix) on lipid and protein packing. Small unilamellar vesicles with radii of curvature of ~10 nm reveal considerable conformational differences between inner and outer monolayers (20). Even in vesicles with radii of 20 nm these differences lead to distinct differences in molecular packing (21). Such differences in packing should lead to differences in composition and the possibility of lateral segregation of lipids into different domains in highly curved bilayers has been suggested (22). Hence the second consequence of the formation of a hernia should be the formation of a region of altered composition (Fig. 2).

Phospholipid structure and composition and enzyme activity

In eukaryotic cells, the colocalization of particular proteins and phospholipids is a well-established phenomenon (e.g., 23, 24). In *E. coli*, the localization of the penicillin binding proteins (PBPs) to particular membrane domains has also been suggested. These proteins direct the synthesis of peptidoglycan with one PBP, PBP3, essential for septation and others, such as PBP2, required for elongation (25, 26). The localization of different PBPs in different membrane domains is suggested by their associations with different subsets of inner membrane vesicles after electrophoretic separation (27). The authors propose that these associations reflect the existence of domains within the membrane specifically concerned with cell elongation and septation. Similar asymmetries in the lipid and protein content of *E. coli* vesicles have also been observed after sucrose gradient centrifugation. Other fractionation studies suggest a large amount

of all PBPs in a labile structure that may correspond to the periseptal annuli (28), while immuno-electronmicroscopy suggests that PBP1b is localized in small regions that may correspond to adhesion sites (29).

Enzyme activity as well as localization is also sensitive to phospholipid structure and composition (for review see reference 30). This is exemplified by the enzymes synthesizing the precursors of peptidoglycan. The repeating unit of *E. coli* peptidoglycan consists of *N*-acetylglucosamine (NAcGlc) linked to *N*-acetylmuramic acid (NAcMur) pentapeptide (31, 32). The polysaccharide strands formed have β-(1–4) links between the sugars and are 30 dissaccharide units (30 nm) long on average. The strands are also cross-linked between the short peptides attached to NAcMur. The assembly of the repeating unit takes place on a lipid carrier, bactoprenyl phosphate (33). The first reaction of the bactoprenyl cycle is the transfer of *P*-NAcMur pentapeptide from UDP-NAcMur pentapeptide to bactoprenyl phosphate with the release of UMP. The second reaction is the transfer to NAcGlc from UDP-NAcGlc to bactoprenyl-*P*-*P*-NAcMur pentapeptide to form the disaccharide pentapeptide repeating unit. The first reaction is catalyzed by phospho-NAcMur pentapeptide translocase and the second reaction by UDP-NAcGlc:NAcMur-(pentapeptide)-*P*-*P*-bactoprenyl-*N*-AcGlc transferase. Hence these enzymes play a key role in the initial stages of peptidoglycan synthesis. Both appear sensitive to their lipid microenvironment.

The solubilized translocase from *Micrococcus luteus* was stimulated by the addition of a neutral lipid fraction (34). The translocase from *Staphylococcus aureus* also shows a dependence on lipids and has been studied in detail by Neuhaus and co-workers. Firstly, they established the microenvironment of the fluorescent lipid intermediate, bactoprenyl-*N*-AcMur-(N^{ϵ}-dansyl)-pentapeptide, with a variety of physical techniques (35); secondly, they perturbed the physical state of the membrane lipids by temperature changes and treatment with *n*-butanol (36, 37). For example, low concentrations (120–180 mM) of *n*-butanol increased the fluidity of the microenvironment of the enzyme and stimulated its transfer activity by 65%; concentrations outside this range inhibited the enzyme. The authors concluded that "the physical state of the lipid matrix has a major effect on the catalytic activity of the translocase" (36). A similar relationship between n-butanol concentration and translocase activity was also observed by Strominger and co-workers (38). The lipid dependence of the translocase from *E. coli* has also been examined (39); in this case it was suggested that phospholipids sensitive to phospholipase A_2 and D are necessary for enzymic activity.

The second enzyme in the bactoprenyl cycle, the transferase, also has an activity stimulated by a crude lipid extract (40) and it has been speculated that this activity in vivo may require a lipid microenvironment similar to that of the translocase (32).

Finally, the synthesis of the lipid carrier, bactoprenyl phosphate, is sensitive to lipid composition (32). The phosphokinase responsible for the phosphorylation of bactoprenol in *S. aureus* revealed an activity stimulated by phosphatidylglycerol more than by phosphatidylethanolamine and stimulated more by cardiolipin with saturated fatty acid chains than by cardiolipin with unsaturated chains (41); subsequent studies showed that, in general, activity was stimulated by lipids that provided a hydrated, loosely packed, highly fluid environment (42).

A relationship between autolysin activity and phospholipid composition is also fundamental to the hernia model. It is therefore encouraging that the activity of a peptidoglycan hydrolase of *E. coli*, *N*-acetylmuramyl-L-alanine amidase, responds in vitro and in vivo to its phospholipid environment (43). Enzyme activity was increased by phospatidylglycerol concentrations $\leq 50\ \mu M$, decreased by phosphatidylglycerol concentrations $> 300\ \mu M$, and unaffected by cardiolipin; according to the authors, these results reflect an in vivo control of amidase activity by the surrounding phosphatidylglycerol.

The Gram-negative sacculus must be more than one layer thick (at least temporarily or locally)

After the reinforcement of a weakened area at a hernia, the sacculus is probably thicker than a single layer. The body of evidence supporting multilayered peptidoglycan (5, 44–49) includes direct evidence from neutron small angle scattering on isolated sacculi of *E. coli* that 75–80% is single layered and 20–25% is triple layered (50); no evidence was found for a region with a double layer or with more than three layers. The implication for the hernia model is that the formation of a hernia must stimulate the immediate synthesis of a triple layer of reinforcing peptidoglycan.

Growth of the peptidoglycan sacculus in patches

The subunits of peptidoglycan are disaccharides that polymerize to form the glycan chains. Each subunit has one short peptide that may cross-link to the peptide of another subunit to make the network of glycan strands cross-linked by dimeric peptide bridges that constitutes peptidoglycan. The fate of these bridges differs considerably depending on whether they are made up of tetramer–trimers or tetramer–tetramers (5). The tetra–tri dimers represent cross-linkage between old and new peptidoglycan and most, if not all, are turned over in a generation. The tetra–tetra dimers represent cross-linkage both between new and new and between new and old peptidoglycan and only half are turned over per generation. One interpretation is that tetra–tri dimers specifi-

cally cross-link one layer with another while the tetra–tetra dimers cross-link both within and between layers (5). These findings have been accommodated into the "patches" model in which the synthesis and attachment of a non-stress–bearing patch of peptidoglycan to the sacculus is followed by the cleavage of the old stress-bearing bonds (5).

The hernia model also predicts growth in such patches but with the difference that cleavage precedes synthesis. Since the majority of glycan strands are only 5–10 dissacharides long, albeit with a higher average (51), the minimum size for a hernia may correspond to the dimensions of these strands (see Appendix) and hence determine whether one (52, 53) or two (52, 54) strands are inserted. Such strands are probably inserted along the circumference of a circle in the plane of the short axis of the cell (54), suggesting each hernia may also have its long axis in this latitudinal direction.

Contractile proteins should interact with the peptidoglycan of the hernia

In the eukaryote *Dictyostelium discoideum*, the capping of surface receptors and accompanying changes in cortical tension are generated by the contractile protein, myosin (55). In both *Dictyostelium* and lymphocytes, specific receptors on the cell surface bind to the tetravalent lectin, concanavalin A; these form a patch on the cell surface and then a cap at one end of the cell; an increase in cortical tension accompanies the capping. In *Dictyostelium*, myosin is essential for both capping the receptors and the cortical stiffening. A "rigor" contraction of a cortical shell of actin and myosin is also believed to underlie the stiffening and change in cell shape after the depletion of cellular ATP. After studies of myosin-minus *Dictyolstelium* and of the location of myosin in wild-type cells during chemotaxis, it has been suggested that the stimulation of one edge of the cell with chemoattractant results in a local breakdown of the actin–myosin network and the consequent extension of a pseudopod (55). In the yeast *Saccharomyces cerevisiae*, studies of myosin-defective mutants show that myosin is required for the correct localization and deposition of chitin and cell wall components during cell growth and division (56, 57).

In addition to the actomyosin cytoskeleton, microtubules and intermediate filaments interact with the membranes of eukaryotic cells (58). Evidence for such elements in prokaryotes supports a role for contractile proteins in the maintenance of bacterial shape. Polypeptides with physical properties characteristic of actin and myosin have been described in various bacteria, including *E. coli* (59–62). Tubulin- and actin-like proteins have been described in archaebacteria after antibody cross-reactivity and inhibitor studies (63). In *E. coli*, we have identified a 180-kD protein that shows strong immunological cross-reactivity with a myosin heavy chain from both chicken smooth muscle and yeast (64). Recently, another 180-kD protein, MukB, has been found to be involved in chromosome segregation in *E. coli* (65); this protein reveals an intriguing similarity to a contractile protein, dynamin, which is involved in the organization of both microtubules and endocytosis in eukaryotes (66, 67). Despite the reservations of Koch (68), it does therefore appear that *E. coli* possesses the elements, if not the entirety, of a eukaryotic cytoskeleton.

To constrain the contraction to the hernia, the model requires that contractile proteins in the cytoplasm interact with membrane proteins that bind to peptidoglycan only in the region of the hernia (see Appendix). The binding protein therefore acts as an anchor point for the contractile apparatus. The other anchor point is a cytoskeletal structure. Although formally possible, it is not proposed that the peptidoglycan-binding proteins actually correspond to the contractile proteins. Hence, by directing the contractile apparatus to a region of specific lipid structure and composition, the hernia confers "intelligence" on contractile proteins.

Cleavage of excess oligopeptidoglycan bonds by autolysins projecting either outward from the cytoplasmic membrane or inward from the outer membrane

After the reinforcement of peptidoglycan at the site of a hernia, the sacculus at this site is then thicker than normal. According to the hernia model, a class of autolysins anchored in either the cytoplasmic or outer membranes degrade the peptidoglycan in the newly reinforced and thickened region. Given that 20 amino acids can span, for example, the cytoplasmic membrane, some 7–9 nm wide, and that the periplasm (in which the peptidoglycan lies) is around 12 nm wide (11), the active site of such an autolysin could easily be located in the peptidoglycan (Fig. 3).

DISCUSSION

The hernia model offers several advantages over existing models. Firstly, it relies on known mechanisms. Radii of curvature are known to be related to phospholipid structure and composition (13, 15, 21, 22) and phospholipid composition is known to affect the location and activity of numerous enzymes, including those involved in peptidoglycan synthesis (37, 39) and hydrolysis (43). Secondly, it avoids the problem of the lysis that should result if weakened regions of the wall are not reinforced preferentially; unlike other models, in the hernia model a weakened wall is more likely to be strengthened than a strong one. Thirdly, it will maintain cylindrical shape. The maintenance of cylindrical shape poses a severe problem

for the patches model; growth in patches as proposed by Holtje and Glauner (5) should lead to a rounding up of the cell (3). In the hernia model, growth in patches is compatible with the maintenance of cylindrical shape because of a factor eschewed by Koch, the existence of contractile proteins (64, 65). In this model, the contraction of envelope-associated, myosin-like proteins ensures the maintenance of cylindrical shape by pulling in the hernia (see Appendix). Autolysins then operate in this region to cleave excess peptidoglycan and so prevent an increase in local diameter: the truss is removed.

Several predictions follow from the model. The initial membrane-associated steps in peptidoglycan are executed by the phospho-NAcMur-pentapeptide translocase and the UDP-*N*-AcGlc:*N*-AcMur-(pentapeptide)-*P*-*P*-bactoprenyl-*N*-Ac-Glc transferase; hydolysis is mediated, in part, by *N*-acetylmuramyl-L-alanine amidase. The activity or partitioning of these enzymes should be shown to depend on the radius of curvature of micelles. The feasability of this approach is demonstrated by studies of the activity of protein kinase C; activity was found to be dependent on micelle formation as determined by the length of the fatty acid chains of phosphatidylcholine (69). Periodically curved bilayer structures as short as 15 nm have been described in the plasma membrane of the bacterium *Streptomyces hygroscopicus* and attributed to lipid segregation (70); such curved regions might constitute precursors of hernias. It is also tempting to speculate that the Bayer bridges (12, 71), junctions between the inner and outer membranes, correspond to hernias: direct evidence for the existence of hernias may come from improved techniques of electron microscopy. The study of peptidoglycan composition in mutants in which phospholipid composition has been altered may prove particularly rewarding. Altering the balance of phosphatidylglycerol to phosphatidylethanolamine or of saturated to unsaturated fatty acids should affect the formation of hernias; small angle neutron scattering of the corresponding sacculi should reveal an alteration in the proportion of the triple-layered structure. This structure should be absent after increases in the osmolarity of the growth medium sufficient to prevent the formation of hernias in mutants defective in osmoregulation. Finally, *E. coli* mutants defective in the contractile protein required for shape maintenance should be spherical with thicker peptidoglycan.

Membrane phospholipids demonstrate a variety of physical properties including lateral and transverse asymmetry, curvature, and the formation of bilayer and nonbilayer structures. Such properties may play important roles in important biological processes and, in the hernia model, we invoke one of these properties, curvature, in the control of wall synthesis. Since such control is required by both eukaryotic and prokaryotic cells, the model may prove of general relevance.

APPENDIX

The purpose of this appendix is to attempt an approximate quantification of the effects discussed in the paper. Although much more detailed and complex models could, and hopefully will, be developed, it is believed that the present discussion at least confirms the feasibility of the proposed hernia model.

The first topic discussed is the size of hernias. This is approached by first reviewing available data on the mechanical properties of lipid bilayers, and then discussing the formation of two possible shapes of hernia resisted by two different forms of mechanical behavior. After this, energy requirements for contractile proteins are considered by calculating the work required to pull in a hernia. Finally, the time scale of these events is discussed to estimate the number of hernias required and their life span.

Mechanical properties of lipid bilayers

The model used for the mechanical properties of a lipid bilayer is derived from Bloom et al. (72). Essentially the membrane is assumed to be a linear elastic material whose stiffness generates stresses in response to in-plane stretching and to bending. It is assumed, however, to be effectively a "two-dimensional liquid" so that there are no shear stresses in the plane of the membrane. As a result tensile forces in the membrane are equal in all directions and given by

$$N = K_a \Delta A/A, \tag{1}$$

where N is tensile force per unit width, A is original surface area of unstretched membrane, ΔA is increase in surface area due to stretching, and K_a is elastic constant, typically in the range 0.1–1.0 N/m.

If the membrane is curved as well as stretched then the above calculation applies to the average of what is happening in the two layers, while the extra stretching of one layer compared with the other gives the bending. On the same argument as above, the bending moment (M) at a point is uniform in all directions and is related to the two principal radii of curvature at the point (R_1 and R_2, say) by

$$M = k_c(1/R_1 + 1/R_2), \tag{2}$$

where k_c is an elastic constant for which a typical value would be 10^{-19} N-m The bending moments will be accompanied by shear forces normal to the plane of the membrane, which will cause shear distortions. Data for shear stiffness does not appear to be readily available and Bloom et al. allow for it (approximately) in the value of k_c.

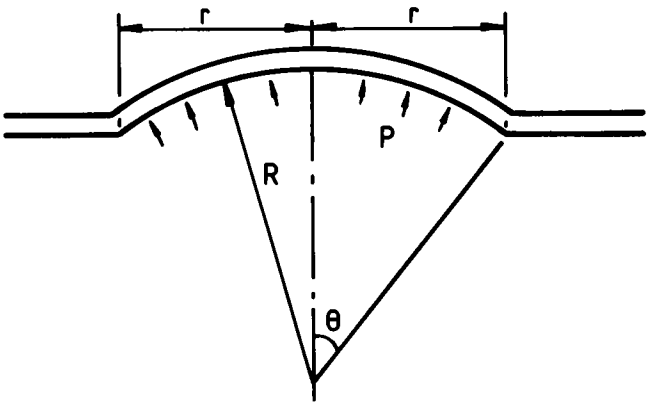
When the hernia forms, both bending and stretching action can be expected to resist the internal pressure. However, with time, changes to the compositions of the monolayers, with lipids with large head groups accumulating on the outside of curves and those with small head groups on the inside, will lead to the membrane becoming naturally curved and so the bending stresses will diminish and the turgor pressure will be increasingly resisted by tensile stresses.

Circular hernia due to stretching action

The first simple model assumes that the hernia forms on a circular area of flat unstretched membrane, radius r, and bending effects are ignored. Under these assumptions the membrane curves into a dome, radius R, say, as shown in Fig. A1. The tensile force N is uniform throughout. It is assumed that only material initially within the circular area forms the hernia so that

$$A = \pi r^2 \tag{3}$$

If this assumption is incorrect, the effect of lipids being drawn into the hernia will be to reduce the stiffness K_a. It seems reasonable to suppose



that this must happen to some extent since there is no obvious mechanism to resist the force N at the edge of the hernia.

Now the surface area of the hernia is given by

$$A + \Delta A = 2\pi R^2(1 - \cos\theta), \tag{4}$$

and

$$\sin\theta = r/R. \tag{5}$$

Combining Eqs. 1, 3, 4, and 5 gives

$$N = K_a(2 - 2\cos\theta - \sin^2\theta)/\sin^2\theta. \tag{6}$$

This membrane stress will be able to resist a turgor pressure p if

$$p = 2N/R, \tag{7}$$

and hence

$$(pr/2K_a) = (2 - 2\cos\theta - \sin^2\theta)/\sin\theta$$
$$= 2\sin^3(\theta/2)/\cos(\theta/2). \tag{8}$$

For small values of θ, i.e., relatively flat hernias we can use the approximations

$$pr/2K_a \approx \theta^3/4, \tag{9}$$

and

$$\theta \approx r/R, \tag{10}$$

whence

$$R \approx r^{2/3}(K_a/2p)^{1/3}. \tag{11}$$

Taking $K_a = 0.3$ N/m and $p = 3 \times 10^5$ N/m^2 gives

$$R = 8r^{2/3}, \tag{12}$$

where R and r are measured in nm.

Long hernia due to stretching action

To test the sensitivity of the value for radius of curvature to the assumption about the shape of the hernia, we can consider another simple case, that of a long thin hernia. Away from the ends it will form into a cylindrical surface. If the width is $2b$, and 2θ is again the angle subtended by the hernia, then

$$A \text{ (per unit length)} = 2b$$
$$A + \Delta A = 2R\theta, \tag{13}$$
$$\sin\theta = b/R, \tag{14}$$

and hence

$$N = K_a(\theta - \sin\theta)/\sin\theta. \tag{15}$$

For this case the pressure p is related to N by

$$p = N/R = N\sin\theta/b. \tag{16}$$

Hence

$$pb/K_a = \theta - \sin\theta. \tag{17}$$

For small angles

$$pb/K_a = \theta - (\theta - \theta^3/3! + \cdots) \approx \theta^3/6, \tag{18}$$

and

$$R \approx b/\theta, \tag{19}$$

so

$$R = b^{2/3}(K_a/6p)^{1/3}. \tag{20}$$

Using the same values of K_a and p as before gives

$$R = 5.5b^{2/3} \tag{21}$$

for R and b in nanometers, showing that the radius of curvature is not very sensitive to the shape of the hernia.

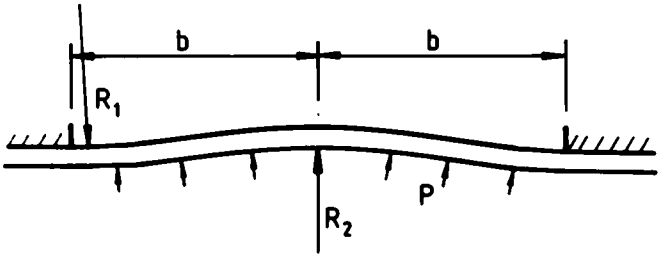
Long hernia due to bending action

The effect of ignoring bending is that at the edge of the hernia there is a sharp kink of theoretically zero radius, which would be prevented in practice by the bending stiffness of the membrane. The alternative simple analysis is to consider bending alone. Assuming that the hernia remains relatively flat, the case of the long hernia can be dealt with using standard results from engineering bending theory. If the edge of the hernia is assumed to approximate to a fixed condition, then standard results (see e.g., reference 73) are that the bending moments at the edge and center are $pb^2/3$ and $pb^2/6$, respectively, and that the minimum radii of curvature are at these positions, and are given by

$$R_1 = 3k_c/pb^2 \quad R_2 = 6k_c/pb^2, \tag{22}$$

as shown on Fig.A2.

This analysis assumes that the membrane remains relatively flat; more precisely, that if v measures the displacement from the initial shape (assumed to be flat) then $dv/dz \ll 1$, where z is the coordinate shown in Fig. A2. The limit that this imposes can be determined as



follows. From Roark (73), the variation of bending moment with z is given by

$$M = pb\left(z - \frac{z^2}{2b} - \frac{b}{3}\right). \quad (23)$$

Hence points of zero moment are given by solving

$$3z^2 - 6bz + 2b^2 = 0, \quad (24)$$

$$\text{i.e.} \quad \frac{z}{b} = 1 \pm \sqrt{3} = 1.577 \quad \text{or} \quad 0.423. \quad (25)$$

The deflected shape is given by

$$v = \frac{P}{24k_c}(4bz^2 - 4b^2z^2 - z^2), \quad (26)$$

$$\frac{dv}{dz} = \frac{P}{24k_c}(12bz^2 - 8b^2z - 4z^3), \quad (27)$$

$$= \frac{1.54pb^3}{24k_c}, \quad (28)$$

at the point of zero moment, which will also be points of maximum gradient. For $p = 3 \times 10^5$ N/m^2 and $k_c = 10^{-19}$ N-m, this gives

$$\frac{dv}{dz} = 0.19 \times 10^{-3}\, b^3,$$

for b in nanometers. Hence this theory is appropriate if b is less than, say 10 nm. Above that it will be increasingly approximate.

Assuming the theory to be valid, and taking the above numerical values gives

$$R_1 = 1000/b^2 \quad R_2 = 2000/b^2, \quad (29)$$

for R and b in nanometers. Thus bending action suggests that the radius is large for small b and declines toward zero as b increases, whereas stretching action shows the radius increasing as b increases. Equating R_2 and R (Eqs. 21 and 29) shows that the two curves cross at $b = 9.2$ nm, $R = 24$ nm, suggesting that for hernias around this size the pressure is resisted by a combination of stretching and bending, while one mode dominates elsewhere. The value of R found (24 nm) indicates the order of magnitude of curvature that can be obtained.

Work required to pull in hernia

Considering a hernia with a circular base, radius 10 nm, the foregoing calculations for stretching effects indicate a radius of curvature of $8 \times 10^{2/3} = 37$ nm, corresponding to an angle θ of 15.6°. The volume of a hernia of spherical shape is given by

$$\tfrac{1}{3}\pi R^3(2 - 3\cos\theta + \cos^3\theta) \approx \pi R^3\theta^4/4 \quad \text{for small } \theta.$$

For the case in point the volume is 220 nm^3, and hence the work required to pull in the hernia against the turgor pressure of 3×10^5 N/m^2 is $3 \times 10^5 \times 220 \times 10^{-27} = 6.5 \times 10^{-20}$ J. Part of this can be contributed by the tension in the membrane doing work as it relaxes back to its original unstressed state; on the other hand, selective diffusion of different types of lipid will have given the hernia a natural curvature which the contractile protein will have to oppose. Hydrolysis of ATP to ADP per molecule = 5×10^{-20} J, so 1 ATP could be sufficient.

Number of hernias

Since the average glycan chain contains 30 subunits and there are some $4–5 \times 10^6$ subunits per cell, around 10^5 hernias will be required per generation providing each hernia inserts only one glycan strand. Assuming a generation time of 10^3 seconds, the minimum number of hernias that must be created per second is therefore 100.

Lifespan of hernias

Assuming hernias to have a circular base, radius 10 nm, then the base area is ~300 nm^2. If the total area of the membrane is 10^7 nm^2, and assuming that there are 100 contractile proteins, then the probability of a hernia containing a protein is 3×10^{-3}. If there are n hernias, then on average there should be $3 \times 10^{-3} \times n$ hernias containing proteins, and this should remain constant as proteins diffuse randomly through the membrane.

The average distance, s, that a protein moves from its starting point in time t, is given by

$$s = (4Dt)^{1/2}, \quad (30)$$

where D is a diffusion coefficient for which a typical value would be 3×10^{-10} cm^2 s^{-1} (3×10^{-4} nm^2 s^{-1}). Hence the time taken for a protein in a hernia to diffuse out of it will on average be of the order of

$$t = r^2/4D = 10^2/4 \times 3 \times 10^4 \approx 10^{-3}s.$$

The rate at which proteins leave hernias is therefore $3 \times 10^{-3} \times n/10^{-3}$ per second = $3n$ per second and so $3n$ proteins must enter hernias per second in a steady state, and $3n$ hernias per second will be pulled in. If 100 hernias are created per second then putting $3n = 100$ gives $n = 33$, i.e., there must be 33 hernias in existence at any one time. On average their life span will be 33×10^{-3} s.

Typical diffusion coefficients for lipids are a factor of 100 greater than that given above, so the exchange of lipids in and out of the hernia is fast relative to the movement of proteins.

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