**REVIEW
ARTICLE****A hypothetical holoenzyme involved in the replication of the murein sacculus of *Escherichia coli***

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Keywords: morphogenesis, cell elongation and division, murein, three-for-one growth strategy, trimeric cross-bridges**Growth and division of the murein sacculus**

The bag-shaped murein sacculus is a unique biopolymer consisting of glycan strands (poly-MurNAc-GlcNAc) cross-linked by short peptides (Fig. 1) (Rogers *et al.*, 1980; Höltje & Schwarz, 1985; Glauner *et al.*, 1988; Labischinski & Maidhof, 1994; Koch, 1995). It is part of the bacterial cell wall and may be looked at as a kind of exoskeleton that endows the cell with mechanical strength and its genetically determined specific shape. The murein sacculus has to be replicated into two daughter sacculi during the cell cycle by a mechanism that guarantees maintenance of mechanical stability and shape.

A hypothetical model will be presented that could explain growth and division of the stress-bearing murein sacculus of *Escherichia coli* by a safe and precise mechanism. It is proposed that a multienzyme complex consisting of murein hydrolases and synthases copies the existing murein sacculus that plays the role of a template. Thus, the specific shape of a bacterium may be transmitted to progeny cells by direct heritage of structural information.

The rod-shaped murein sacculus of *E. coli* can be described as composed of a cylindrical middle part and two hemispherical polar caps. The shape of the cell is therefore defined by the diameter and the length of the cylindrical part of the rod. Whereas the length of the cell doubles in one generation, the diameter stays constant during steady-state growth (Donachie *et al.*, 1976; Trueba & Woldringh, 1980). Cell division at the midpoint of the cell gives rise to two identical new rod-shaped daughter cells (Fig. 2). Hence, a mechanism that describes the replication of the three-dimensional structure of the murein sacculus has to explain only three things. Firstly, how the diameter is kept constant, secondly, how the length is doubled in one generation, and thirdly, how the cell finds its midpoint to correctly localize cell division.

Murein, the shape-maintaining structure of a bacterium

The murein of *E. coli* forms a thin, basically monolayered sacculus (Labischinski *et al.*, 1991). Its chemistry is known in quite some detail since the establishment of a method that determines the muropeptide composition by HPLC (Glauner *et al.*, 1988). The structure of the murein as shown in Fig. 1 turned out to contain not only dimeric peptide-cross-bridges that connect two glycan chains, but in addition trimeric cross-bridges that link together three chains (Glauner *et al.*, 1988). Such oligomeric muropeptide structures can be formed because of the chemistry of the peptide bridges that are the result of a transpeptidation reaction between a donor peptide and an acceptor peptide. The donor peptide must be a pentapeptide, whereas the acceptor peptide just has to present a free ϵ -amino group of the diaminopimelic acid (A_{2pm}). By cleaving off the terminal D-Ala residue, the pentapeptide plays the role of an energy donor for the formation of the DD-peptide bond between the carboxyl group of the penultimate D-Ala of the donor peptide and the ϵ -amino group of the A_{2pm} in the acceptor peptide. As a result, a dimeric cross-bridge is formed in which the peptide that functioned as a donor still carries a free ϵ -amino group at its A_{2pm} residue. Hence the dimeric cross-bridge can function as an acceptor in another transpeptidation reaction. A second such transpeptidation would yield a trimeric cross-bridge. Again the donor peptide in the trimeric structure will maintain its free ϵ -amino group and therefore could play the role of an acceptor in yet another transpeptidation. In *E. coli*, muropeptides up to tetramers have been found, but structures combining more than four glycan strands could not be detected. Probably, they do not exist for sterical reasons.

The trimeric muropeptide structure is of particular interest because the three glycan strands at this cross-linking element cannot be arranged in one layer except for the case where ends of glycan strands are cross-linked. The trimeric cross-bridge and the two different types of

Fig. 1. Structure of the murein of *E. coli*. To indicate that the peptide moieties protrude in different directions (forming an angle of 90°), different letters were used: standard for peptides arranged in the plane of the drawing, italic and bold for peptides directing downward, and italic and not bold for peptides directing upward; the structure shown in grey is located in a layer below the structure shown in black; A₂pm, diaminopimelic acid.

Fig. 2. Schematic representation of the morphogenesis of *E. coli* during the cell cycle.

dimeric cross-bridges (Fig. 1), namely one that consists of two tetra-peptides (Tetra-Tetra) and one that is formed by one tri- and one tetra-peptide (Tetra-Tri), are key structures in the growth model described below.

The metabolism of the sacculus during growth has been analysed by pulse and pulse-chase experiments (Burman *et al.*, 1983; Goodell & Schwarz, 1983, 1985; Goodell, 1985; Driehuis & Wouters, 1987; de Jonge *et al.*, 1989; Glauner & Höltje, 1990; Höltje & Glauner, 1990; Romeis *et al.*, 1991; Obermann & Höltje, 1994). Two results were particularly surprising and need to be explained by any hypothetical growth model. Firstly, the finding that per generation about 40–50 % of the material of the murein sacculus is released during growth and fed into an efficient recycling process (Goodell & Schwarz, 1985; Goodell, 1985; Park, 1993, 1995). Secondly, the fact that all trimeric cross-bridges and all of the dimeric cross-bridges between tri- and tetrapeptides, but not those consisting of two tetra-peptides, are cleaved during one generation (Glauner & Höltje, 1990). Some interesting models for growth of the murein sacculus of *E. coli* and *Salmonella* have been presented (Burman & Park, 1984; Cooper *et al.*, 1988; de Jonge *et al.*, 1989; Cooper, 1991; Koch, 1990, 1995), but none of these can explain all of the experimental results.

Towards a growth mechanism

It is difficult to envisage a safe mechanism for the enlargement of a stress-bearing structure that would not follow the strategy of first attaching new material to the

Fig. 3. Murein enlargement according to the 'three-for-one' growth model. The rods represent the glycan strands. A₂pm, diaminopimelic acid.

structure to be enlarged before the sacculus under stress is cleaved to allow the integration of the new material into the stress-bearing layer. Thus, any growth model better obeys the principle of 'make-before-break' (Koch *et al.*, 1982; Koch, 1990). A molecular consideration of the predominantly monolayered murein of *E. coli* (Labischinski *et al.*, 1991) suggested a simple possible mechanism (Höltje, 1993). Three glycan strands cross-linked to one another are covalently attached underneath one strand, which functions as a docking strand, in the stress-bearing layer by transpeptidation to the dimeric cross-bridges on both sides of this docking strand (Fig. 3). Trimeric cross-bridges are thus formed, which represent the attachment sites of the new material. Specific removal of the docking strand leads to the insertion of the three new strands into the layer under stress due to the surface tension. For every three new strands inserted, one old strand is removed. Therefore the mechanism has been named 'three-for-one'. Since the glycan strands are arranged perpendicular to the long axis of the rod-shaped cell (Verwer *et al.*, 1978), this will result in cell elongation. The three-for-one mechanism explains both murein turnover and the short half-life and low abundance of trimeric cross-bridges. In addition, the action of the murein hydrolases, potentially autolytic enzymes (Höltje, 1995), would be restricted to sites protected by the added new material, since trimeric cross-bridges would function as specific structural signals.

To avoid disruption of the murein net upon release of the docking strand, a certain arrangement of the cross-linking peptides has to be postulated. The peptides of the docking strand have to be acceptor peptides forming cross-bridges

with donor peptides at the neighbouring glycan strands to the right and left. As explained above, cross-linked donor peptides are characterized by the presence of a free ϵ -amino group at the A₂pm. It is to these free amino groups in the dimeric cross-bridges to which the triple pack of new murein has to be covalently linked. This is done by transpeptidation of the pentapeptidyl donor moieties present in the two outer strands of the triplet to the free ϵ -amino groups of the A₂pm residues in the donor peptide parts of the dimeric cross-bridges that connect the docking strand with the neighbouring strands. Thereby the peptide bonds that are going to be cleaved to release the docking strand will be enclosed by the two new covalent bonds through which the new material is attached (see Fig. 3). It is this arrangement of acceptor and donor peptide moieties in the cross-bridges that connect docking and non-docking strands, that allows the release of the docking strand after the attachment of new murein without interruption of a continuous murein layer. The former donor parts of the dimeric cross-bridges that function as acceptors for the attachment of the triplet are transformed into acceptor peptides because they lose their free ϵ -amino group. The incoming triple pack will also be composed of a non-docking, a docking and a non-docking strand to maintain the alternating sequence of donor and acceptor peptides in the connecting cross-bridges.

The three-for-one mechanism can explain not only longitudinal growth of the sacculus but also constriction. For septum formation to take place, the attachment of new triplets of murein has to be restricted to the midpoint of the cell. Inward growth of a septum could then be achieved either simply by increasing the rate of addition

Fig. 4. Murein constriction according to the 'three-for-one' growth model. The rods represent the glycan strands, the lines the peptides.

of new triplets as compared to hydrolysis of the docking strands, or by the help of a mechanical device such as the FtsZ ring structure (Bi & Lutkenhaus, 1991), which could function to pull the membrane inwards. The ingrowing septum will consist of three layers of murein, with the outer ones forming the two new polar caps after specific removal of the inner layer that consists of docking strands only (Fig. 4). Release of material from the septum during its cutting has previously been proposed to occur also in Gram-positive bacteria (Giesbrecht *et al.*, 1976).

Synthesis of the murein triplets

Pulse-chase studies on the incorporation of new subunits into the existing murein sacculus revealed that new material is exclusively cross-linked with old material (Burman *et al.*, 1983; Cooper *et al.*, 1988; de Jonge *et al.*, 1989; Glauner & Höltje, 1990). This indicates that single strands are inserted rather than cross-linked multiple strands (Koch, 1988). Therefore, we have to assume that the triplet of new murein that is going to be inserted following the three-for-one mechanism is formed in two steps (Fig. 5). We postulate that the middle strand of the triplet is synthesized independently as a kind of primer strand, which then in a second step is completed to the triple pack by the attachment of two additional strands. Synthesis of primer murein that is further modified has earlier been postulated by Wientjes & Nanninga (1991). Following this mechanism, and consistent with the experimental results, newly synthesized material will always be cross-linked with old murein. By way of contrast, during septum formation (see Fig. 4), triplets of new murein are stacked on to each other, thus new material is cross-linked with new material. Indeed this has been shown by pulse-chase experiments with synchronously growing cultures (de Jonge *et al.*, 1989).

Fig. 5. Formation of the murein triplet. (a) A single murein strand that functions as a primer is supplemented to a murein triplet by the synthesis and cross-linkage of two additional strands on both sides of the primer strand. (b) The murein triplet is then covalently attached underneath a docking strand in the murein layer. (c) Upon removal of the docking strand the murein triplet is inserted into the stress-bearing murein. Glycan strands are represented by rods, donor peptides by arrows, and acceptor peptides by lines.

Coordination by formation of a multienzyme complex

Following the three-for-one growth mechanism, several different enzymic reactions must be coordinated. Firstly, the synthesis of a murein triplet, secondly the covalent attachment of the triplet to the stress-bearing murein layer, and finally, the specific hydrolysis of the docking strand. A model for the duplication of the murein sacculus should therefore explain the proper cooperation of murein hydrolases and synthases. Studies on the biochemistry of the enzymes involved in the metabolism of the murein sacculus revealed the presence of multifold murein-synthesizing and murein-hydrolysing enzymes (Höltje & Schwarz, 1985; Shockman & Höltje, 1994). Importantly, protein-protein interactions between both classes of enzymes have recently been demonstrated by affinity chromatography (Romeis & Höltje, 1994). Interestingly, such an interaction has, among others, been shown for a group of murein-hydrolysing enzymes, called lytic transglycosylases (Höltje *et al.*, 1975; Ehlert *et al.*, 1995) that, by starting at one end, degrade the glycan strands in a

Fig. 6. Hypothetical multienzyme complex of a murein replicase holoenzyme. The stress-bearing murein layer is shown schematically in a side view with the glycan strands (running perpendicular to the plane of the drawing) indicated by black dots and the peptide bridges by thick lines. The triplet of new murein consisting of a primer strand (light-grey dot) and two additional strands (dark-grey dots) is synthesized by a dimer of a transglycosylase/transpeptidase bifunctional enzyme (TG/TP) and covalently attached underneath the docking strand by a transpeptidase dimer (TP). Upon release of the docking strand by the action of an endopeptidase (EP) and a lytic transglycosylase (LT), the three new glycan strands are inserted into the stress-bearing layer as shown in the lower panel of the drawing.

processive manner (Beachey *et al.*, 1981). It therefore is tempting to speculate that the polymerization reaction of new murein strands may be directly coupled to a processive degradation of the docking strand by forming an enzyme complex consisting of murein synthases and lytic transglycosylases.

To coordinate the different enzymic steps it is assumed that a multienzyme complex, a murein replicase holoenzyme, is formed. The holoenzyme complex not only completes the cross-linked triplet of glycan strands, but also covalently hooks it by transpeptidation underneath a docking strand, and concomitantly hydrolyses the docking strand to achieve insertion of the new material into the stress-bearing layer of the sacculus. It is proposed that a complex of two murein synthase dimers are combined with two murein hydrolases in a murein replicase holoenzyme, which may assemble on a pre-existing murein primer strand (Fig. 6). A dimer of a bifunctional murein synthase such as penicillin-binding protein (PBP) 1b would synthesize the two outer glycan strands and also would cross-link them to the primer strand that becomes the middle strand of the murein triplet. Dimerization of PBPs 1a and 1b has been demonstrated *in vitro* (Zijderveld *et al.*, 1991). The middle strand functions exclusively as an acceptor, whereas the outer strands function as donors during the formation of

the triplet and the covalent attachment underneath the stress-bearing murein layer. The attachment is accomplished by transpeptidation of the outer strands of the triplet to the free amino groups of the dimeric cross-bridges to the left and right of the docking strand. The reaction would be catalysed by a transpeptidase PBP dimer. It would be this PBP that determines the specificity of the holoenzyme to function as either elongation or septation machinery. It is postulated that two different holoenzymes exist. The specificity of both is due to the presence of either PBP2, which catalyses synthesis of the cylindrical murein (Park & Burman, 1973), or PBP3, which is responsible for septum formation (Botta & Park, 1981). Again, experimental indication for a dimerization of PBP3 does exist (Ayala *et al.*, 1994). The synthase subcomplex would have to associate with the hydrolase subcomplex to form the functional holoenzyme. Affinity chromatography using immobilized lytic transglycosylases demonstrated that besides the bifunctional murein transglycosylases/transpeptidases PBP 1a and 1b and the transpeptidases PBP2 and 3, the endopeptidases PBP4 and PBP7 were also specifically retained by immobilized lytic transglycosylases (Romeis & Höltje, 1994; von Rechenberg *et al.*, 1996). Therefore, we conclude that the hydrolase subcomplex of the holoenzyme consists of a lytic transglycosylase and an endopeptidase. Such a

Fig. 7. Replication of the murein sacculus. The vertical lines represent the glycan strands with the thick ones representing docking strands. The horizontal lines represent acceptor peptides and the horizontal arrows donor peptides. The cylindrical part of the murein sacculus is spread out in the plane of the drawing. The murein of the young cell in the upper part consists of 24 glycan strands, 12 of which are docking strands, which will be released during growth and replaced by 36 new strands. The murein of the cell shown in the lower part is made of twice as much murein (48 strands) as the upper cell and is twice as long ($2b$), although the circumference (a) is the same.

combination of enzyme specificities could completely degrade the docking strand to its monomeric subunits.

Replication of the murein sacculus

Identical duplication of the murein sacculus requires that the diameter is maintained, that the length is doubled in one generation, and that the division takes place precisely at the midpoint of the cell. Because of the arrangement of the glycan strands perpendicular to the long axis of the rod (Verwer *et al.*, 1979), maintenance of the circumference of the cell as well as a doubling in length could easily be achieved when every second murein ring structure formed by a given number of murein glycan strands is replaced by three new rings, each one consisting of the same number and lengths of murein glycan strands that formed the released ring. It is therefore proposed that the existing murein sacculus serves as a template (matrix) for the synthesis of new murein.

By moving along the docking strand the multienzyme complex can precisely copy the length of the docking strand, which will function as a template for the synthesis of the two outer strands of the murein triplet. Although these two strands will have exactly the same length as the

original strand, this is not the case for the primer strand, which has been synthesized independently. Therefore, the length of the primer must be adjusted by a kind of proofreading step. If too long, the ends of the strand cannot be cross-linked to the neighbouring strands and hence may be recognized by exo-muramidases that remove these free ends. Such a formatting process has indeed been shown to occur (Glauner & Höltje, 1990). Furthermore, since the enzyme complex will maintain the pattern of cross-linkage, the degree of cross-linkage will remain unchanged as wall growth preceeds.

Assuming that docking and non-docking strands alternate in the murein sacculus, then the insertion of three new strands for each of the existing docking strands will result in a doubling of the amount of murein (Fig. 7). An exact doubling in murein would, however, depend on the replacement of only the old docking strands that were present after the last cell division, i.e. at the beginning of the new cell cycle and not the ones newly inserted during growth. Thus, it has to be postulated that the enzymes can discriminate between old and newly inserted docking strands. There seems indeed to be a simple way how this could be accomplished. Pulse-chase experiments have shown that newly synthesized murein is cross-linked

exclusively by Tetra–Tetra cross-bridges (Glauner & Höltje, 1990). If we assume that strands linked by Tetra–Tetra cross-bridges cannot function as docking strands unless the Tetra–Tetra bridges are altered to Tetra–Tri cross-links by the action of a LD-carboxypeptidase, there would be a means to control doubling of murein. We postulate that during or immediately after cell division all existing cross-bridges are trimmed to Tetra–Tri structures, allowing growth according to the three-for-one mechanism. However, the Tetra–Tetra cross-links in newly inserted material remain unchanged and therefore a docking strand in newly inserted material cannot be replaced. Since the Tetra–Tri cross-bridges are steadily replaced during growth by Tetra–Tetra cross-links, the murein sacculus will be turned into a metabolically inert structure at the end of the cell cycle. Growth therefore comes to a halt until all cross-bridges are again modified to Tetra–Tri structures by a pulse in LD-carboxypeptidase activity. Synthesis of the septum may not depend on the presence of Tetra–Tri cross-bridges. A fluctuation in LD-carboxypeptidase activity has been demonstrated in synchronously growing cultures of *E. coli* (Beck & Park, 1976). According to this mechanism, the cell is able to measure when it has doubled in length. This proposal is supported by the finding of pulse-chase experiments (Glauner & Höltje, 1990) that all Tetra–Tri but not Tetra–Tetra cross-bridges are cleaved during one generation.

With the glycans running perpendicular to the longitudinal axis of the rod-shaped sacculus, the copying mechanism will yield a sacculus twice as long as the template but with the diameter kept constant. Formation of a septum at the midpoint of the cell will finally give rise to the formation of two identical daughter cells, which are also identical to their mother cell. We are left with the question, how can the cell find its midpoint? It is not clear whether the cell in each generation defines the site of division *de novo* or whether this site is pre-determined. Besides chemical means, simple physical factors have also been suggested to be involved (Nanninga, 1988; Koch & Höltje, 1995). It thus becomes clear that the fundamental questions of the morphogenesis of a simple organism such as a bacterial cell, while still not understood, may finally be explained by a simple molecular mechanism.

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REFERENCES

- Ayala, J. A., Garrido, T., de Pedro, M. A. & Vicente, M. (1994). Molecular biology of bacterial septation. In *Bacterial Cell Wall*, pp. 73–101. Edited by J.-M. Ghuyssen & R. Hakenbeck. Amsterdam: Elsevier.
- Beachey, E. H., Keck, W., de Pedro, M. A. & Schwarz, U. (1981). Exoenzymatic activity of transglycosylase isolated from *Escherichia coli*. *Eur J Biochem* **116**, 355–358.
- Beck, B. D. & Park, J. T. (1976). Activity of three murein hydrolases during the cell division cycle of *Escherichia coli* K-12 as measured in toluene-treated cells. *J Bacteriol* **126**, 1250–1260.
- Bi, E. & Lutkenhaus, J. (1991). FtsZ ring structure associated with cell division in *Escherichia coli*. *Nature* **354**, 161–164.
- Botta, G. A. & Park, J. T. (1981). Evidence for involvement of penicillin-binding protein 3 in murein synthesis during septation but not during cell elongation. *J Bacteriol* **145**, 333–340.
- Burman, L. G. & Park, J. T. (1984). Molecular model for elongation of the murein sacculus of *Escherichia coli*. *Proc Natl Acad Sci USA* **81**, 1844–1848.
- Burman, L. G., Reichler, J. & Park, J. T. (1983). Evidence for multisite growth of *Escherichia coli* murein involving concomitant endopeptidase and transpeptidase activities. *J Bacteriol* **156**, 386–392.
- Cooper, S. (1991). *Bacterial Growth and Division*. San Diego, CA: Academic Press.
- Cooper, S., Hsieh, M.-L. & Guenther, B. (1988). Mode of peptidoglycan synthesis in *Salmonella typhimurium*: single strand insertion. *J Bacteriol* **170**, 3509–3512.
- Donachie, W. D., Begg, K. J. & Vicente, M. (1976). Cell length, cell growth and cell division. *Nature* **264**, 328–333.
- Driehuis, F. & Wouters, J. (1987). Effect of growth rate and cell shape on the peptidoglycan composition in *Escherichia coli*. *J Bacteriol* **169**, 97–101.
- Ehlert, K., Höltje, J.-V. & Templin, M. F. (1995). Cloning and expression of a murein hydrolase lipoprotein from *Escherichia coli*. *Mol Microbiol* **16**, 761–768.
- Giesbrecht, P., Wecke, J. & Reinicke, B. (1976). On the morphogenesis of the cell wall of staphylococci. *Int Rev Cytol* **44**, 225–318.
- Glauner, B. & Höltje, J.-V. (1990). Growth pattern of the murein sacculus of *Escherichia coli*. *J Biol Chem* **265**, 18988–18996.
- Glauner, B., Höltje, J.-V. & Schwarz, U. (1988). The composition of the murein of *Escherichia coli*. *J Biol Chem* **263**, 10088–10095.
- Goodell, E. W. (1985). Recycling of murein by *Escherichia coli*. *J Bacteriol* **163**, 305–310.
- Goodell, E. W. & Schwarz, U. (1983). Cleavage and resynthesis of peptide cross-bridges in *Escherichia coli* murein. *J Bacteriol* **156**, 136–140.
- Goodell, E. W. & Schwarz, U. (1985). Release of cell wall peptides into culture medium by exponentially growing *Escherichia coli*. *J Bacteriol* **162**, 391–397.
- Höltje, J.-V. (1993). ‘Three for one’ – a simple growth mechanism that guarantees a precise copy of the thin, rod-shaped murein sacculus of *Escherichia coli*. In *Bacterial Growth and Lysis: Metabolism and Structure of the Bacterial Sacculus*, pp. 419–426. Edited by M. A. de Pedro, J.-V. Höltje & W. Löffelhardt. New York & London: Plenum Press.
- Höltje, J.-V. (1995). From growth to autolysis: the murein hydrolases in *Escherichia coli*. *Arch Microbiol* **164**, 243–254.
- Höltje, J.-V. & Glauner, B. (1990). Structure and metabolism of the murein sacculus. *Res Microbiol* **141**, 75–89.
- Höltje, J.-V. & Schwarz, U. (1985). Biosynthesis and growth of the murein sacculus. In *Molecular Cytology of Escherichia coli*, pp. 77–119. Edited by N. Nanninga. London: Academic Press.
- Höltje, J.-V., Mirelman, D., Sharon, N. & Schwarz, U. (1975). Novel

- type of murein transglycosylase in *Escherichia coli*. *J Bacteriol* **124**, 1067–1076.
- de Jonge, B. L. M., Wientjes, F. B., Jurida, J., Driehuis, F., Wouters, J. T. M. & Nanninga, N. (1989).** Peptidoglycan synthesis during the cell cycle of *Escherichia coli*: composition and mode of insertion. *J Bacteriol* **171**, 5783–5794.
- Koch, A. L. (1988).** Biophysics of bacterial walls viewed as stress-bearing fabric. *Microbiol Rev* **52**, 337–353.
- Koch, A. L. (1990).** Additional arguments for the key role of ‘smart’ autolysins in the enlargement of the wall of Gram-negative bacteria. *Res Microbiol* **141**, 529–541.
- Koch, A. L. (1995).** *Bacterial Growth and Form*. New York: Chapman & Hall.
- Koch, A. L. & Höltje, J.-V. (1995).** A physical basis for the precise location of the division site of rod-shaped bacteria: the Central Stress Model. *Microbiology* **141**, 3171–3180.
- Koch, A. L., Higgins, M. L. & Doyle, R. J. (1982).** The role of surface stress in the morphology of microbes. *J Gen Microbiol* **128**, 927–945.
- Labischinski, H. & Maidhof, H. (1994).** Bacterial peptidoglycan: overview and evolving concepts. In *Bacterial Cell Wall*, pp. 23–38. Edited by J.-M. Ghuysen & R. Hakenbeck. Amsterdam: Elsevier.
- Labischinski, H., Goodell, E. W., Goodell, A. & Hochberg, M. L. (1991).** Direct proof of a ‘more-than single-layered’ peptidoglycan architecture of *Escherichia coli* W7: a neutron small-angle scattering study. *J Bacteriol* **173**, 751–756.
- Nanninga, N. (1988).** Growth and form in microorganisms: morphogenesis of *Escherichia coli*. *Can J Microbiol* **34**, 381–389.
- Obermann, W. & Höltje, J.-V. (1994).** Alterations of murein structure and of penicillin-binding proteins in minicells from *Escherichia coli*. *Microbiology* **140**, 79–87.
- Park, J. T. (1993).** Turnover and recycling of the murein sacculus in oligopeptide permease-negative strains of *Escherichia coli*: evidence for an alternative permease system and for a monolayered sacculus. *J Bacteriol* **175**, 7–11.
- Park, J. T. (1995).** Why does *Escherichia coli* recycle its cell wall peptides? *Mol Microbiol* **17**, 421–426.
- Park, J. T. & Burman, L. G. (1973).** FL 1060: a new penicillin with a unique mode of action. *Biochem Biophys Res Commun* **51**, 863–868.
- von Rechenberg, M., Ursinus, A. & Höltje, J.-V. (1996).** Affinity chromatography as a means to study multi-enzyme-complexes involved in murein synthesis. In *Microbial Drug Resistance*, vol. 2, pp. 155–157. Edited by A. Tomasz. Larchmont, NY: Mary Ann Liebert.
- Rogers, H. J., Perkins, H. R. & Ward, J. B. (1980).** *Microbial Cell Walls and Membranes*. London & New York: Chapman & Hall.
- Romeis, T. & Höltje, J.-V. (1994).** Specific interaction of penicillin-binding proteins 3 and 7/8 with the soluble lytic transglycosylase in *Escherichia coli*. *J Biol Chem* **269**, 21603–21607.
- Romeis, T., Kohlrausch, U., Burgdorf, K. & Höltje, J.-V. (1991).** Murein chemistry of cell division in *Escherichia coli*. *Res Microbiol* **142**, 325–332.
- Shockman, G. D. & Höltje, J.-V. (1994).** Microbial peptidoglycan (murein) hydrolases. In *Bacterial Cell Wall*, pp. 131–166. Edited by J.-M. Ghuysen & R. Hakenbeck. Amsterdam: Elsevier.
- Trueba, F. J. & Woldringh, C. L. (1980).** Changes in cell diameter during the division cycle of *Escherichia coli*. *J Bacteriol* **142**, 869–878.
- Verwer, R. W. H., Nanninga, N., Keck, W. & Schwarz, U. (1978).** Arrangement of glycan chains in the sacculus of *Escherichia coli*. *J Bacteriol* **136**, 723–729.
- Wientjes, F. B. & Nanninga, N. (1991).** On the role of the high molecular weight penicillin-binding proteins in the cell cycle of *Escherichia coli*. *Res Microbiol* **142**, 333–344.
- Zijderveld, C. A. L., Aarsman, M. E. G., den Blaauwen, T. & Nanninga, N. (1991).** Penicillin-binding protein 1B of *Escherichia coli* exists in dimeric forms. *J Bacteriol* **173**, 5740–5746.