Polysaccharide Biosynthesis

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ABSTRACT Polysaccharide synthesis is discussed from the point of view of the sources of biological information that determine the structures and control the rates of synthesis of complex polysaccharides. It is concluded that three types of information contribute in important and different ways, namely enzyme specificity, primer substances, and the structure of the cytoplasmic membrane. Each of these factors is discussed in a general way with examples of its contribution to the structure and organization of specific polysaccharides.

The general problem of polysaccharide synthesis may be considered from a number of points of view. It is possible, for instance, to divide polysaccharides into groups on the basis of chemical complexity and it would be feasible to discuss homopolysaccharides, polysaccharides with simple repeating units, and complex heteropolysaccharides as three distinct problems of biosynthesis. Alternatively, it would be possible to separate polysaccharides into functional groups and consider as separate topics the synthesis of intracellular storage polysaccharides, cell wall structural polysaccharides, and extracellular or capsular polysaccharides. Both approaches are reasonable and it is possible to develop the themes that the biochemical mechanisms of biosynthesis become more complex as one considers polysaccharides with increasing structural complexity or increasing functional complexity. It is obvious that more enzymes will be involved in the formation of the variety of linkages of a complex heteropolysaccharide than will be required to form the one or two types of linkage in a homopolysaccharide. It is also obvious that a complex function may require the intervention of enzymes, cofactors, and control systems to ensure the coordination of polysaccharide synthesis with the formation of other substances, as in the synthesis of the bacterial cell wall, or to regulate synthesis and degradation, as in the case of glycogen formation and breakdown.

A third way of discussing polysaccharide synthesis is to consider the sources of biological information that determine the structure and control the synthesis of polysaccharides. This is the point of view that will be taken in the present paper and it will be assumed that there are primarily three sources of information, namely enzyme specificity, primer substances, and the structure of the cytoplasmic membrane. These sources of information may well be

related to one another. At the present stage of understanding in molecular biology, however, each of these components may be considered as an independent source of information and, in each case, the source of a different type of information. Enzyme specificity derives from protein structure (ultimately from nucleic acid nucleotide sequences) and specific enzymes are responsible for the synthesis of activated monosaccharides, the nucleoside diphosphate sugars, and for the specific transfer of glycosyl residues from these nucleoside diphosphate sugars to acceptor molecules, usually other saccharide residues. Primers may be defined as substances that cannot be derived readily *de novo* from enzymes and metabolites; they serve as enzyme substrates and probably also direct enzyme action by allosteric interactions and structural effects. The cytoplasmic membrane has recently come to the foreground as the site of cell wall polysaccharide synthesis and, as will be mentioned below, a phospholipid that is probably present in the cytoplasmic membrane serves as a coeznyme in the synthesis of at least some cell wall polysaccharides. What determines the structure of the cytoplasmic membrane is at present almost purely a matter of speculation and, in fact, Sonneborn (1) has pointed out that the relationship between the cell genome and the structure and function of the cytoplasmic membrane remains one of the most important unexplored areas of biology. To summarize, therefore, enzyme specificity, primer materials, and the structure of the cytoplasmic membrane may be considered as the three basic sources that determine and control the synthesis of polysaccharides, although all three factors may not operate directly in every case.

The possibility that other sources of information may function in polysaccharide synthesis has been proposed. McMullen (2) has suggested that "nucleoside diphosphate saccharides . . . are analogous to the activated amino acid in protein template biogenesis and correspondingly enter into the same kind of . . . coding mechanisms involving polynucleotide structures. . . ." Such speculation is interesting but, as far as we are aware, has no experimental basis.

Enzyme Specificity

Little comment is required on the major role played by enzyme specificity in determining and controlling polysaccharide synthesis. The synthesis of most polysaccharides begins with the formation of a nucleoside diphosphate glycosyl derivative from a nucleotide triphosphate and a glycosyl phosphate ester. Fig. 1 illustrates the formation of uridine diphosphate glucose (=UDP-glucose) from uridine triphosphate and glucose-1-phosphate. Even at this initial stage the reaction shows a high degree of specificity and control. Bernstein and Robbins (3), for example, studied the formation of the structurally similar nucleotides UDP-glucose and thymidine diphosphate glucose

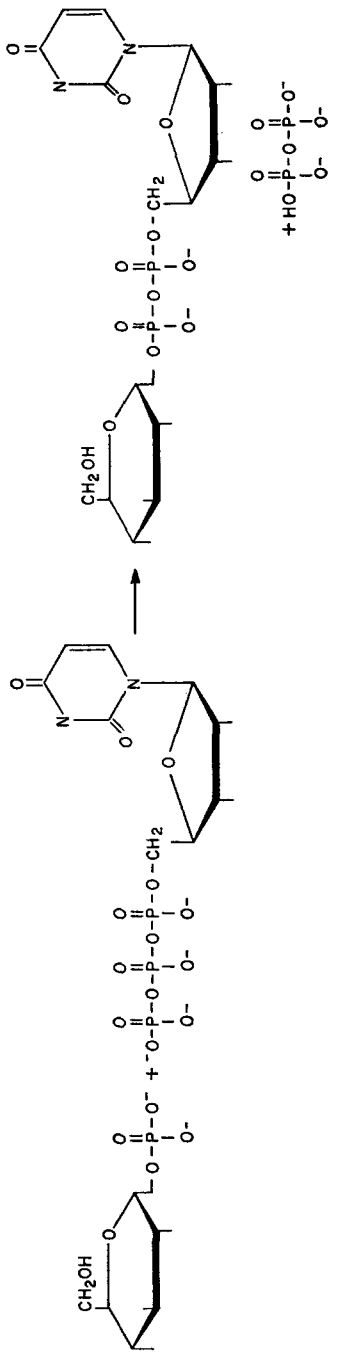

FIGURE 1. Enzymatic synthesis of UDP-glucose from UTP and glucose-1-phosphate.

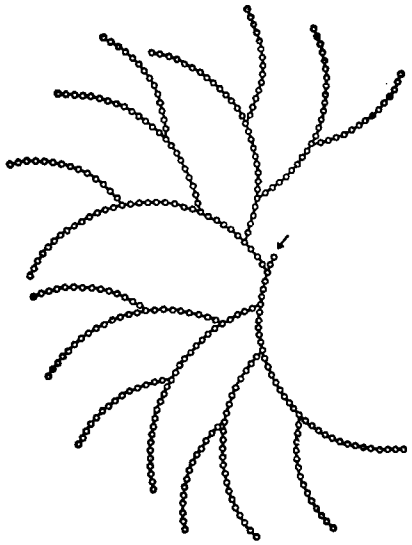

FIGURE 2. Diagrammatic representation of a portion of a glycogen molecule. The open circles represent glucose units. The interresidue linkages are of the α-1,4-glucosidic type and the interchain linkages are of the α-1,6-glucosidic type. The arrow indicates the single reducing end group of the molecule.

(=TDP-glucose) by extracts of enteric bacteria. It was shown that the nucleotides are formed by separate soluble enzymes, each with its own pattern of feed-back control. Approximately sixty nucleoside diphosphate sugars are known at the present time. Some of these are formed directly from an α-D-glycosyl-1-phosphate and a nucleoside triphosphate, but many are products of oxidation, reduction, and epimerization reactions that are catalyzed by specific soluble enzymes. For example, such reactions bring about the transformation of TDP-glucose to TDP-rhamnose, the direct precursor of polysaccharide rhamnose. The known systems are summarized in the reviews by Leloir (4) and Ginsburg (5) and will not be enumerated here.

The enzymes that catalyze the transfer of glycosyl residues from nucleoside diphosphate sugars to the oxygen residues of other saccharide moieties in general show absolute specificity with respect to the linkage position and anomeric configuration. Since, as far as is known, all nucleoside diphosphate sugars have the same absolute configuration (=α-D) at the phosphate-glycosyl linkage some transfer reactions clearly take place with retention of this configuration at the glycosyl position while other transfer reactions lead to inversion of configuration. In either case the energy of the system is preserved, presumably by way of covalent intermediates; thus, it is possible that transfer reactions that take place with retention of the glycosyl carbon atom configuration are actually double inversion reactions. According to this concept (6) the enzyme that will catalyze transfer with retention of configuration reacts with the nucleoside diphosphate sugar with displacement of the nucleoside diphosphate and the formation of an enzyme-sugar covalent intermediate. This intermediate has the inverted glycosyl configuration and reaction with the final acceptor in a second displacement reaction leads to restoration of the original glycosidic configuration. Reactions that involve over-all inversion, on the other hand, would be visualized as simple displacement reactions on the nucleoside diphosphate sugar glycosyl linkage by the acceptor group oxygen moiety. While these simple schemes would account adequately for the occurrence of both types of glycosidic linkages in polysaccharides, little serious work has yet been carried out on the mechanisms of transfer reactions.

The Role of Primer in Polysaccharide Biosynthesis

Although enzymatic specificity adequately accounts for the sequential addition of subunits into correct linkage with growing polysaccharide chains, it appears that the entire assembly of the molecule cannot be achieved by enzymes, coenzymes, and metabolites alone. An interesting example of such an instance is the biosynthesis of glycogen. Glycogen is composed of several hundred to several thousand chains of α-1,4-linked D-glucose units, the interchain linkage being of the α-1,6-glucosidic type (Fig. 2). It is synthe-

sized by the transfer of glucose units from UDP-glucose to the nonreducing end groups of primer glycogen, catalyzed by the enzyme glycogen synthetase (7). The action of the enzyme is to lengthen the exterior chains of the molecule (Fig. 3). Branching of these newly created chains is catalyzed by amylo-1,4 → 1,6-transglucosidase (branching enzyme), the over-all effect of the two enzymes being to cause the outward growth of the molecule. Glycogen itself is the best acceptor or primer in the glycogen synthetase reaction, although oligosaccharides as small as maltose may also act as acceptors, but at a very much reduced rate (8).

The question of how the first glycogen molecules appear in the cell raises two alternatives. Either glycogen is synthesized *de novo* from precursors present in the cell or preformed glycogen is present at the birth of each new cell. *De novo* synthesis by glycogen synthetase does not seem likely at present since

FIGURE 3. Action of glycogen synthetase. The figure shows transfer of glucose from UDP-glucose to the nonreducing end group of a glycogen molecule resulting in chain elongation.

glucose and other common metabolites do not act as acceptors in the reaction. As mentioned previously, this enzyme is able to use maltose as an acceptor at a slow rate, but since maltose and its homologues were shown by Olavarria (9) to be degradation products of glycogen rather than its precursors, this pathway seems unlikely. Brown (10), in fact, has reported that no *de novo* glycogen synthesis can be detected when purified glycogen synthetase is incubated with UDP-glucose in the absence of primer glycogen.

The *de novo* synthesis of glycogen from glucose-1-phosphate by a mixture of highly purified, primer-free phosphorylase and branching enzyme has been reported by Brown et al. (11). It is suggested that the process involves the formation of a glucosyl enzyme which becomes the acceptor for chain growth. However, such a reaction in vivo would require a favorable glucose-1-phosphate to inorganic phosphate ratio which would necessitate compartmentalization of the cell, since this ratio normally favors the phosphorolysis reaction. It has also been observed that the appearance of glycogen in chick embryo precedes by a significant period the appearance of phosphorylase (12). Furthermore, it has been shown that in McArdle's disease, which is characterized by an accumulation of glycogen in muscle, there is a complete absence of phosphorylase (13). Thus from the standpoint of present knowledge the question of *de novo* synthesis of glycogen must be left open. Considering the

problem from the point of view of genetic transfer from one generation of cells to the next, *de novo* synthesis would imply that the information for glycogen synthesis is transmitted by enzyme specificity (as determined ultimately by DNA nucleotide sequences). On the other hand, the requirement for a primer would imply the transmission of the primer substance as information supplementary to that transmitted by DNA.

Biosynthetic pathways for a large number of polysaccharides, in addition to glycogen, have been described, but in no case can the presence of primer be completely excluded, although it seems that in at least two well defined systems, the biosynthesis of hyaluronic acid (14) and paramylon (15), primer may not be necessary.

A more complex product-primer system involves the synthesis of the bacterial cell wall. Partial removal of the cell wall from Gram-negative strains by penicillin or lysozyme treatment leads to protoplasts or L-forms that readily revert to normal bacterial growth following the removal of penicillin or lysozyme. Presumably the fragments of cell wall that remain attached to these protoplasts can function as sites of wall organization. Whether repair and reintegration of damaged wall take place or whether wall fragments serve a more indirect role in the reversion to bacillary form has never been clearly shown. The simplest assumption would be that cell wall fragments prime synthesis and repair in the same way that glycogen primes its own synthesis. In the light of the properties of the "nonreverting" L-forms discussed below, however, the possibility must also be considered that the wall fragments of reverting protoplasts serve only to slow the diffusion of wall macromolecules being lost to the medium or some other indirect physical function. This is an important problem that might be amenable to analysis by a combined biochemical and electron micrograph study.

Complete removal of the cell wall as determined by electron microscopy leads to the formation of stable or nonreverting L-forms that have been studied in detail by Landman and coworkers (16). These preparations grow slowly and may be propagated on soft agar. The agar fibers presumably serve in some way as a substitute for the cell wall. Among the stable L-forms that have been studied those derived from some strains revert to bacillary form very rarely or not at all, while others revert readily if special growth conditions are provided. The most interesting observation is that stable *B. subtilis* L-forms will revert if placed on a hard agar (2.5%) or hard gelatin (30%) surface. Since primer in the form of cell wall fragments is presumably absent in this system, the agar or gelatin must serve to initiate cell wall synthesis by an indirect effect.

A variety of proposals have been made for the mechanism of priming effects in cell wall synthesis, but no definitive judgement can be made on the basis of available data. The primer has been postulated to be cell wall fragments

or other components on or near the cytoplasmic membrane. It has also been postulated that in the absence of a cell wall the various component macromolecules that would make up the cell wall diffuse away from the exterior surface of the membrane too rapidly to undergo "self-organizing" reactions such as covalent cross-linking and complex hydrogen bond interactions. This "diffusion barrier" postulate for the priming function of cell wall in its own synthesis is attractive, but is not compatible with the reversion of stable L-forms on hard agar since agar has little effect on the diffusion rate of even rather large molecules. It is possible that hard agar and hard gelatin change the conformation of the cytoplasmic membrane and that this conformational change leads in some as yet undefined way to the organization of the cell wall macromolecules into a fixed pattern. Certainly it would be useful to know whether the initiation of cell wall synthesis in the hard agar system bears any relationship at all to the resumption of cell wall synthesis in reverting L-forms, since in one case it is logical to assume that priming is a physical effect of the medium while in the other case a chemical priming by wall fragments would seem more reasonable.

The Role of the Cytoplasmic Membrane in Polysaccharide Synthesis

Work with cell-free systems that catalyze the synthesis of heteropolysaccharides by transfer from nucleoside diphosphate sugar derivatives has suggested that elements of the cytoplasmic membrane are involved in the synthetic process. For example, studies by Glaser and Brown (17) with a preparation from Rous sarcoma cells and the work of Dorfman and coworkers (14) with enzymes from Group A *Streptococci* showed that hyaluronic acid synthesis is associated with cell particulate fractions. Smith et al. (18) purified the system that catalyzes the synthesis of Type III *Pneumococcus* polysaccharide by ammonium sulfate fractionation and found that although the final preparation is essentially free of nucleic acid it can be precipitated by ultracentrifugation and exhibits typical "microsomal" properties. Recent work on the biosynthesis of the lipopolysaccharide component of the cell wall of Gram-negative bacteria has provided direct evidence for the involvement of membrane components in complex polysaccharide synthesis. Lipopolysaccharide has a highly complex structure which, as indicated in Fig. 4, can be subdivided into three main components: the backbone and the R-specific side chains, which together constitute the "core," and the smooth O-antigen. The backbone (19, 20) contains heptose, phosphate, *O*-phosphorylethanolamine, and 2-keto-3-deoxy-octonate (21), which links it to the lipid A of Westphal (20). The R-specific chains (19) contain glucose, galactose, and *N*-acetylglucosamine and are probably joined to the backbone by a glucose-heptose linkage (22). The *O*-specific side chains determine the serological groups of enteric bacteria and may be linked to the *N*-acetylglucosamine units of the R-chains

(22). Unlike the core material the smooth O-antigen varies widely in monosaccharide composition. *Salmonella* groups B and E, which will be discussed below, have repeating sequences containing galactose, mannose, and rhamnose. Mutants that lack the capacity to synthesize UDP-glucose or UDP-galactose form an incomplete lipopolysaccharide that is deficient in both the R-specific and O-specific side chains (23, 24). Such mutants have been used by Rothfield, Osborn, and Horecker (25) to study the incorporation of specific glucosyl or galactosyl residues into the incomplete lipopolysaccharide core.

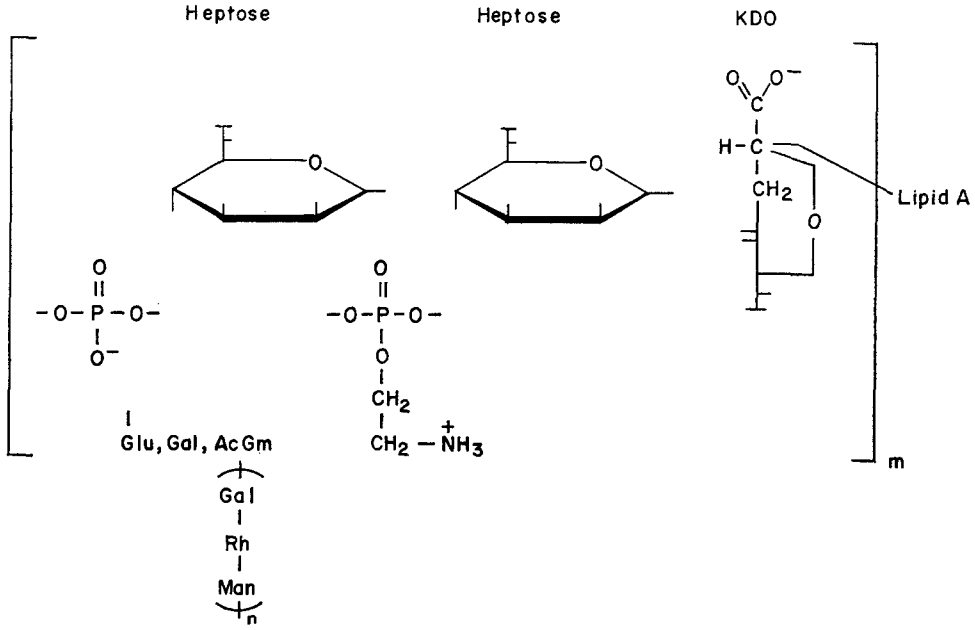

FIGURE 4. Structural components of lipopolysaccharide. The core substance is enclosed by the square brackets and contains 2-keto-3-deoxy-octonate (KDO), heptose, O-phosphorylethanolamine, phosphate, glucose (Glu), galactose (Gal), and *N*-acetylglucosamine (AcGm). The core is linked to lipid A through KDO. The round brackets enclose the repeating sequence of the O-antigen of *Salmonella newington* which contains galactose, rhamnose (Rh), and mannose (Man). The values of *n* and *m* are unknown.

The reactions have been formulated as follows:—

(*a*) Glucose-deficient lipopolysaccharide + UDP-glucose
→ glucosyl-lipopolysaccharide (+ UDP)

(*b*) Glucosyl-lipopolysaccharide + UDP-galactose
→ galactosyl-glucosyl-lipopolysaccharide (+ UDP)

In each case enzyme activity is found in both the cell wall membrane fraction and "soluble" fraction derived from sonicated cells. The substrate specificity and the binding of enzyme to its macromolecular substrate have been

studied by combining the soluble enzymes with various polysaccharide preparations.

Although purified lipopolysaccharide is inactive as a substrate in the transferase reactions, a complex composed of lipid, extracted from the cell envelope fraction by lipid solvents, and purified lipopolysaccharide is an excellent substrate. The active component of the lipid extract was shown to be phosphatidyl ethanolamine. The complex is prepared by an annealing process which suggests that the lipopolysaccharide is partially "dissolved" in the lipid to create an environment suitable for the binding and action of the transferase enzymes. Furthermore the galactose transferase is specifically

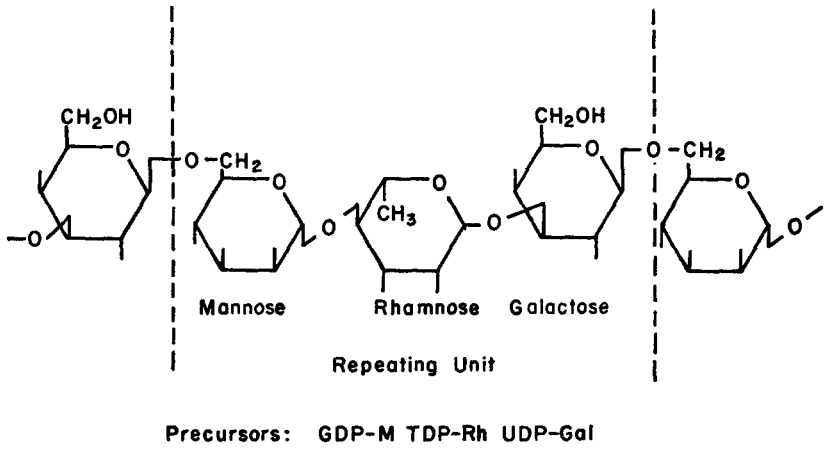

FIGURE 5. Structure of the *Salmonella newington* O-antigen.

adsorbed to the galactose-deficient lipopolysaccharide-lipid complex, the binding being dependent on the presence of both components. A model suggested by these results for the formation of lipopolysaccharide is one in which the transferase enzymes bind specifically to incomplete lipopolysaccharide associated with phospholipid at the surface of the membrane.

The biosynthesis of the core or rough lipopolysaccharide used in the above studies proceeds by the successive transfer of monosaccharide residues from nucleotide sugars to the incomplete lipopolysaccharide. In contrast, the biosynthesis of the O-antigen or smooth polysaccharide component of the lipopolysaccharide is a more complex process. Successful in vitro incorporation of sugars from the nucleotide sugar precursors requires the presence of a complex system which contains the cell wall membrane fraction. Such incorporation has been reported in the case of *S. typhimurium* by Zeleznick et al. (26) and by Nikaido and Nikaido (27) and in the case of *S. newington* by Robbins et al. (28). The O-antigens of *S. typhimurium* and *S. newington* have repeating units containing the same three sugars, mannose, rhamnose, and

galactose (Fig. 5), and the former also contains the dideoxyhexose abequose. In each of these cases the presence of membrane material is necessary for synthesis. Weiner et al. (29) and Wright et al. (30) have also demonstrated that the O-antigen chains are not directly derived from the nucleotide sugars but are assembled from trisaccharide repeating units preformed on a lipid intermediate. The sequence of reactions involved in the biosynthesis of the

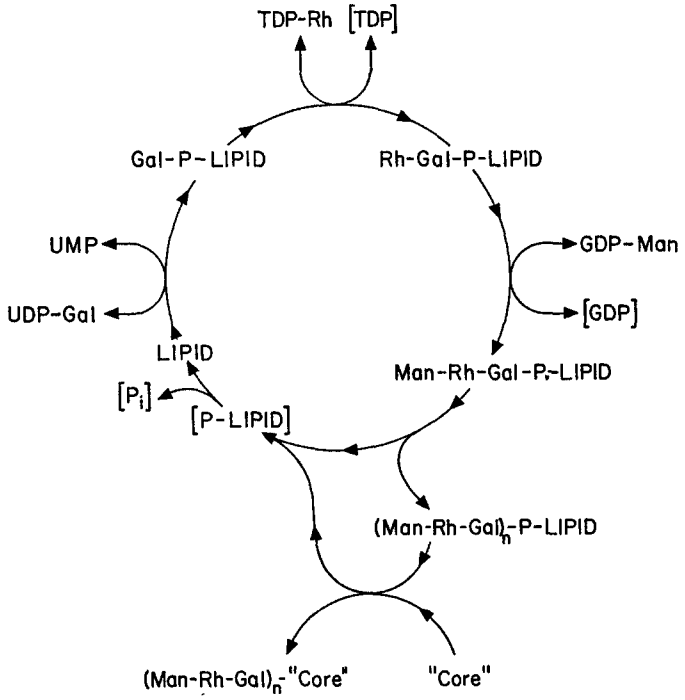

FIGURE 6. Scheme for the involvement of lipid intermediates in the synthesis of *Salmonella* O-antigen. The system is described in the text. Postulated intermediates and products are shown in square brackets. The following abbreviations are used:
UDP-Gal, uridine diphosphate-D-galactose
TDP-Rh, thymidine diphosphate-L-rhamnose
GDP-Man, guanosine diphosphate-D-mannose.

Salmonella O-antigen is shown in Fig. 6. The first transfer to the lipid acceptor is that of galactose-1-phosphate from the donor UDP-galactose with the concurrent release of UMP. UMP inhibits the reaction and can also cause its reversal. The second transfer to the lipid acceptor is that of rhamnose, added to every available galactosyl residue to form the disaccharide intermediate. In the presence of GDP-mannose the trisaccharide repeating unit is formed and immediately converted into polysaccharide. Direct evidence for the participation of the trisaccharide phosphate lipid intermediate at this stage has been provided by Weiner et al. (29). Working with *S. typhimurium*

they isolated and characterized the mannosylrhamnosylgalactose-1-phosphate lipid. These workers also isolated long O-antigen chains terminated by galactose-1-phosphate, a finding which is indicative of the formation of a lipid-linked polysaccharide by a chain-elongating mechanism based on the poly-

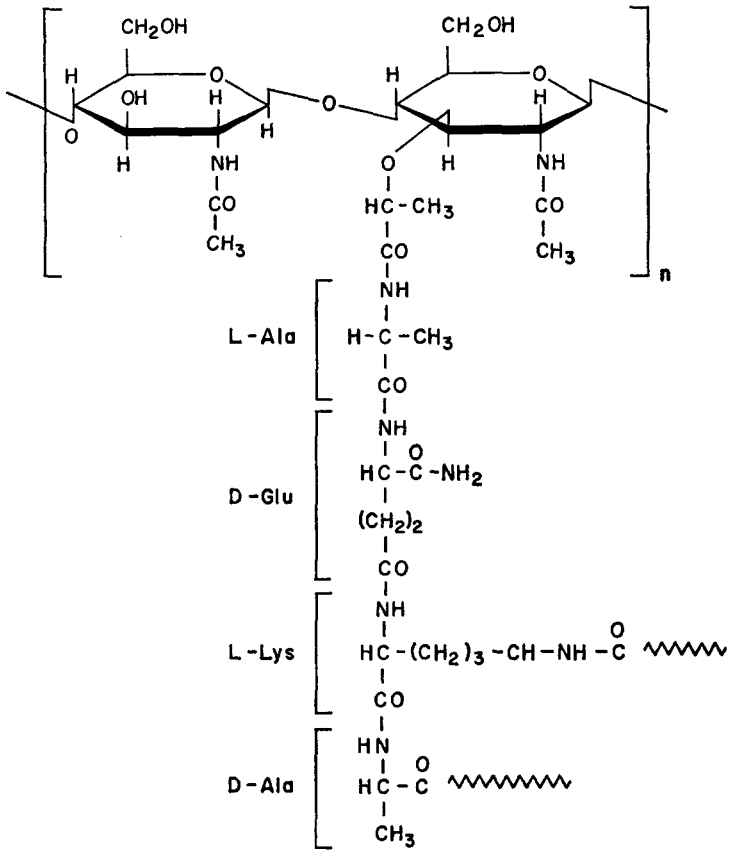

FIGURE 7. Repeating unit of the mucopeptide of Gram-positive bacteria. The polysaccharide backbone has alternating units of *N*-acetylglucosamine and *N*-acetylmuramic acid. The N-acetylmuramic acid is substituted by a tetrapeptide containing L-alanine, D-isoglutamine, L-lysine, and D-alanine. The zigzag lines indicate the points of attachment of interchain bridges.

merization of repeating units provided by the trisaccharide intermediate. The formation of lipopolysaccharide, the final reaction in the sequence, is achieved by a transfer of polysaccharide to the appropriate acceptor in the lipopolysaccharide core.

Such a mechanism has important implications since it illustrates how subunits of the O-antigen, which is exterior to the cell membrane, might be assembled from cytoplasmic precursors in the form of lipid-linked intermediates

in order to facilitate their transport across the membrane. An attractive hypothesis is that the lipid is a component of the membrane closely associated with a series of membrane-bound enzymes forming an organized synthesis and transport system. As will be seen below, a similar mechanism is involved in the formation of the cell wall mucopeptide; thus the reaction may be a general one for the formation of components external to the cytoplasmic membrane.

The mucopeptide consists of a series of polysaccharide chains or "backbone units," with an *N*-acetylglucosaminyl *N*-acetylmuramylpentapeptide repeating sequence (Fig. 7), which are cross-linked to form a continuous, covalently linked network (31). The mucopeptide is external to the cytoplasmic membrane and is responsible for the structural rigidity of the cell wall.

The studies of Chatterjee and Park (32) and Meadow et al. (33) on the biosynthesis of the mucopeptide repeating sequence in Gram-positive bacteria showed that it is formed from the precursors UDP *N*-acetylmuramylpentapeptide and UDP *N*-acetylglucosamine. More recently Strominger and coworkers (34) have shown the direct involvement of membrane-linked lipid intermediates in the formation of mucopeptide components of *S. aureus* and *M. lysodeikticus*. The mucopeptide is formed from the precursors UDP-*N*-acetylmuramylpentapeptide and UDP-*N*-acetylglucosamine by a complex series of reactions which may be summarized as follows:—

Lipid + UDP-*N*-acetylmuramylpentapeptide → lipid-P-*N*-acetyl-muramyl-pentapeptide + UMP

Lipid-P-*N*-acetylmuramylpentapeptide + UDP-*N*-acetylglucosamine → lipid-P-*N*-acetylmuramylpentapeptide-*N*-acetylglucosamine + UDP

Lipid-P-*N*-acetylmuramylpentapeptide-*N*-acetylglucosamine + glycyl-sRNA → lipid-P-*N*-acetylmuramylpentapeptide $(glycine)_n$-*N*-acetylglucosamine + sRNA

Lipid-P-*N*-acetylmuramylpentapeptide $(glycine)_n$-*N*-acetylglucosamine + acceptor → cell wall mucopeptide + lipid + P_i

All the structural subunits of the cell wall mucopeptide are formed on membrane-bound lipid, again emphasizing the central role of lipid intermediates in the formation of components external to the membrane. Strominger and coworkers have isolated lipid-linked intermediates (35) and shown that the acceptor is a glycerol phospholipid to which the disaccharide pentapeptide unit is linked through a pyrophosphate bridge (36).

Preliminary results in our laboratory appear to be consistent with the presence of a pyrophosphate linkage in the intermediates of O-antigen synthesis. Thus, lipid diphosphate sugars would be the membrane-bound counterparts of the nucleoside diphosphate sugars, the lipid moiety being suited to the hydrophobic nature of the membrane. An important question is whether

there are in fact a series of related lipids which have such a function; structural variation of the lipids could provide the specificity necessary for the concurrent formation of the several complex components of the bacterial cell wall. Studies of other membrane-controlled systems, not only in bacteria but in plant and animal cells, should resolve this interesting and important question.

REFERENCES

1. SONNEBORN, T. M., The evolutionary integration of the genetic material into genetic systems, Presented at the Mendel Centennial Celebration at the Genetics Society of America at Fort Collins, Colorado, September, 1965, Madison, University of Wisconsin Press.
2. MCMULLEN, A. I., A template mechanism for the biogenesis of specific polysaccharides, *Excerpta Medica*, International Congress Series No. 77, XIth International Congress of Cell Biology, New York, Excerpta Medica Foundation, 1964, 32.
3. BERNSTEIN, R. L., and ROBBINS, P. W., Control aspects of uridine 5′-diphosphate glucose and thymidine 5′-diphosphate glucose synthesis by microbial enzymes, *J. Biol. Chem.*, 1965, **240**, 391.
4. LELOIR, L. F., Nucleoside diphosphate sugars and saccharide synthesis, *Biochem. J.*, 1964, **91**, 1.
5. GINSBURG, V., Sugar nucleotides and the synthesis of carbohydrates, *Advances Enzymol.*, 1964, **26**, 35.
6. KOSHLAND, D. E., JR., Group transfer as an enzymatic substitution mechanism, *in* A Symposium on the Mechanism of Enzyme Action, (W. D. McElroy and B. Glass, editors), Baltimore, The Johns Hopkins University Press, 1954, 608.
7. LELOIR, L. F., The biosynthesis of polysaccharides, Sixth International Congress of Biochemistry, New York City, Proceedings of the Plenary Sessions, 1964, 15.
8. GOLDEMBERG, S. H., Specificity of uridine diphosphate glucose-glycogen glucosyltransferase, *Biochim. et Biophysica Acta*, 1962, **56**, 357.
9. OLAVARRIA, J. M., The metabolism of oligosaccharides, *J. Biol. Chem.*, 1960, **235**, 3058.
10. BROWN, D. H., in discussion of the paper by L. F. Leloir, Role of uridine diphosphate glucose in the synthesis of glycogen, *in* Ciba Foundation Symposium on Control of Glycogen Metabolism, (W. J. Whelan and M. P. Cameron, editors), Boston, Little, Brown and Company, 1964, 85.
11. ILLINGWORTH, B., BROWN, D. H., and CORI, C. F., The *de novo* synthesis of polysaccharide by phosphorylase, *Proc. Nat. Acad. Sc.*, 1961, **47**, 469.
    BROWN, D. H., ILLINGWORTH, B., and CORI, C. F., The mechanism of the *de novo* synthesis of polysaccharide by phosphorylase, *Proc. Nat. Acad. Sc.*, 1961, **47**, 479.
12. GRILLO, T. A. I., and OZONE, K., Uridine diphosphate glucose-glycogen synthetase activity in the chick embryo, *Nature*, 1962, **195**, 902.
13. SCHMID, R., ROBBINS, P. W., and TRAUT, R. R., Glycogen synthesis in muscle lacking phosphorylase, *Proc. Nat. Acad. Sc.*, 1959, **45**, 1236.

14. MARKOVITZ, A., and DORFMAN, A., Synthesis of capsular polysaccharide (hyaluronic acid) by protoplast membrane preparations of group A *Streptococcus*, *J. Biol. Chem.*, 1962, **237**, 273.
15. MARECHAL, L. R., and GOLDEMBERG, S. H., Uridine diphosphate glucose-β-1,3-glucan β-3-glucosyltransferase from *Euglena gracilis*, *J. Biol. Chem.*, 1964, **239**, 3163.
16. LANDMAN, O. E., and HALLE, S., Enzymically and physically induced inheritance changes in *Bacillus subtilis*, *J. Mol. Biol.*, 1963, **7**, 721.
17. GLASER, L., and BROWN, D. H., The enzymatic synthesis *in vitro* of hyaluronic acid chains, *Proc. Nat. Acad. Sc.*, 1955, **41**, 253.
18. SMITH, E. E. B., MILLS, G. T., BERNHEIMER, H. P., and AUSTRIAN, R., The synthesis of type III pneumococcal capsular polysaccharide from uridine nucleotides by a cell-free extract of *Diplococcus pneumoniae* Type III, *J. Biol. Chem.*, 1960, **235**, 1876.
19. OSBORN, M. J., ROSEN, S. M., ROTHFIELD, L., ZELEZNICK, L. D., and HORECKER, B. L., Lipopolysaccharide of the Gram negative cell wall: biosynthesis of a complex heteropolysaccharide occurs by successive addition of specific sugar residues, *Science*, 1964, **145**, 783.
20. LUDERITZ, O., STAUB, A. M., and WESTPHAL, O., Immunochemistry of O and R antigens of *Salmonella* and related Enterobacteriaceae, *Bact. Rev.*, in press.
21. HEATH, E. C., and GHALAMBOR, M. A., 2-keto-3-deoxy-octonate, a constituent of cell wall lipopolysaccharide preparations obtained from *E. coli*, *Biochem. and Biophysic. Research Comm.*, 1963, **10**, 340.
22. NIKAIDO, H., Biosynthesis of cell wall polysaccharide in mutant strains of *Salmonella*. III. Transfer of L-rhamnose and D-galactose, *Biochemistry*, 1965, **4**, 1550.
23. NIKAIDO, M., Studies on the biosynthesis of cell wall polysaccharide in mutant strains of *Salmonella*. I and II, *Proc. Nat. Acad. Sc.*, 1962, **48**, 1337, 1542.
24. OSBORN, M. J., ROSEN, S. M., ROTHFIELD, L., and HORECKER, B. L., Biosynthesis of bacterial lipopolysaccharide. I. Enzymatic incorporation of galactose in a mutant strain of *Salmonella*. *Proc. Nat. Acad. Sc.*, 1962, **48**, 1831.
25. ROTHFIELD, L., OSBORN, M. J., and HORECKER, B. L., Biosynthesis of bacterial lipopolysaccharide. II. Incorporation of glucose and galactose catalyzed by particulate and soluble enzymes in *Salmonella*, *J. Biol. Chem.*, 1964, **239**, 2788.
ROTHFIELD, L., and HORECKER, B. L, The role of cell-wall lipid in the biosynthesis of bacterial lipopolysaccharide, *Proc. Nat. Acad. Sc.*, 1964, **52**, 939.
ROTHFIELD, L., and TAKESHITA, M., The role of cell envelope phospholipid in the enzymatic synthesis of bacterial lipopolysaccharide: binding of transferase enzymes to a lipopolysaccharide complex, *Biochem. Biophysic. Research Comm.*, 1965, **20**, 521.
26. ZELEZNICK, L. D., ROSEN, S. M., SALTMARSH-ANDREW, M., OSBORN, M. J., and HORECKER, B. L., Biosynthesis of bacterial lipopolysaccharide. IV. Enzymatic incorporation of mannose, rhamnose, and galactose in a mutant strain of *Salmonella typhimurium*, *Proc. Nat. Acad. Sc.*, 1965, **53**, 207.
27. NIKAIDO, H., and NIKAIDO, K., Biosynthesis of cell wall polysaccharide in mutant

strains of *Salmonella*. IV. Synthesis of S-specific side-chain, *Biochem. and Biophysic. Research Commun.*, 1965, **19**, 322.

28. ROBBINS, P. W., WRIGHT, A., and BELLOWS, J. L., Enzymatic synthesis of the *salmonella* O-antigen, *Proc. Nat. Acad. Sc.*, 1964, **52**, 1302.
29. WEINER, I. M., HIGUCHI, T., ROTHFIELD, L., SALTMARSH-ANDREW, M., OSBORN, M. J., and HORECKER, B. L., Biosynthesis of bacterial lipopolysaccharide. V. Lipid-linked intermediates in the biosynthesis of the O-antigen groups of *Salmonella typhimurium*, *Proc. Nat. Acad. Sc.*, 1965, **54**, 228.
30. WRIGHT, A., DANKERT, M., and ROBBINS, P. W., Evidence for an intermediate stage in the biosynthesis of the *Salmonella* O-antigen, *Proc. Nat. Acad. Sc.*, 1965, **54**, 235.
31. SALTON, M. R. J., The Bacterial Cell Wall, New York, Elsevier Publishing Company, 1964, 133.
32. CHATTERJEE, A. N., and PARK, J. T., Biosynthesis of cell wall mucopeptide by a particulate fraction from *Staphylococcus aureus*, *Proc. Nat. Acad. Sc.*, 1964, **51**, 9.
33. MEADOW, P. M., ANDERSON, J. S., and STROMINGER, J. L., Enzymatic polymerization of USP-acetylmuramyl. L-Ala. D-Glu. L-Lys. D-Ala. D-Ala and UDP acetylglucosamine by a particulate enzyme from *Staphylococcus aureus* and its inhibition by antibiotics, *Biochem. and Biophysic. Research Comm.*, 1964, **14**, 382.
34. ANDERSON, J. S., MATSUHASHI, M., HASKIN, M. A., and STROMINGER, J. L., Lipid-phosphoacetylmuramyl-pentapeptide and lipid-phosphodisaccharide-pentapeptide: Presumed membrane transport intermediates in cell wall synthesis, *Proc. Nat. Acad. Sc.* 1965, **53**, 881.
    MATSUHASHI, M., DIETRICH, C. P., and STROMINGER, J. L., Incorporation of glycine into the cell wall glycopeptide in *Staphylococcus aureus:* Role of sRNA and lipid intermediates, *Proc. Nat. Acad. Sc.*, 1965, **54**, 587.
35. ANDERSON, J. S., and STROMINGER, J. L., Isolation and utilization of phospholipid intermediates in cell wall glycopeptide synthesis, *Biochem. and Biophysic. Research Commun.*, 1965, **21**, 516.
36. DIETRICH, C. P., MATSUHASHI, M., and STROMINGER, J. L., Glycerol diphosphate disaccharide-pentapeptide: A functional group of the lipid intermediate in cell wall glycopeptide synthesis, *Biochem. and Biophysic. Research Commun.* 1965, **21**, 619.

Discussion

Dr. Zacharias Dische: I should like to make a comment on the remarks that the Chairman made at the beginning of the second part of this session and relate them to the question of polysaccharides. I think a very important point concerning the structure of polysaccharides which mostly is not considered because of technical difficulties is the existence of trace constituents in the polysaccharides. Here the analytical methods are the limiting factor and for this reason these constituents are very easily over-

looked and yet they may play a very important role. An example of that has been, I think, provided in the last 2 or 4 years by the work of Rodén in Dorfman's laboratory who has shown that the acid mucopolysaccharides—I don't remember whether hyaluronic acid also, but certainly chondroitin sulfuric acid and heparin—are attached to small protein peptides by a handle consisting of one molecule of xylose and two molecules of galactose. So you can have a molecule of about 300,000 which is attached to protein by one single molecule of xylose and this xylose is absolutely necessary to combine these polysaccharides to the protein. I think that such minute trace constituents in polysaccharides will probably be found to be of much greater importance than we at present realize, and that they are prone to be overlooked due to the complex architecture of such polysaccharides as the *Salmonella* polysaccharides. But I think this problem is one of the most important if, out of the chemistry of the polysaccharides, results of the kind Dr. Luria envisions should eventuate.

Dr. Luria: If there are no other questions, we proceed to the last paper in the program. This really brings us to what I think is the essential problem of building the functional organization of the cell. The problem is how to keep things together in such a way that they are going to work at the right time and at the right place, and only into the kind of relations in which they are going to be useful for the specific purpose of the cell. This leads us to what I would call the biochemistry of the future, that is, the biochemistry of cellular membranes. It is fit that the biochemistry of the future should come last in a symposium of the past. Dr. Kennedy has very kindly agreed to open the future for us in closing the symposium.