Direction of Chain Growth in Polysaccharide Synthesis

Work on a bacterial polysaccharide suggests that elongation can occur at the "reducing" end of growing chains.

P. W. Robbins, D. Bray, M. Dankert, A. Wright

The biosynthesis of all four major classes of biological polymer—proteins, nucleic acids, lipids, and polysaccharides—occurs by the sequential addition of subunits to the growing chain. In all four classes the energy for the polymerization is provided by prior attachment of the subunits by a labile linkage to an activating group. Release of his group occurs as the subunit is added to the polymer. Thus a polymer of amino acids is synthesized from amino-acyl sRNA precursors and a polymer of nucleoside monophosphates, from nucleoside triphosphates. In any biosynthesis of this type, two general mechanisms of growth are possible, as is shown in Fig. 1. Each successive addition of a subunit can occur with release of either the activating group attached to the growing chain or that attached to the subunit being added. Either the end of the nascent polymer is transferred to the next subunit or the end of each subunit is transferred to the polymer. For the proteins and lipids that have been studied there are good reasons to believe that the second alternative (*B*) in Fig. 1 represents the actual mechanism of chain growth. In these proteins and lipids it is the activated end of the polymer and not that of the subunit that is transferred.

In contrast, the known mechanisms for polysaccharide synthesis indicate that the first alternative (*A*) in Fig. 1 is operative; in the case of homopolysaccharides the monosaccharide subunits activated at their reducing ends are added in a stepwise fashion to the nonreducing end of the growing polymer. In most cases monosaccharides are "activated" by the formation of nucleoside diphosphate derivatives from which they are transferred to the "nonactivated" growing polymer. In heteropolysaccharide synthesis, little information is available on the mechanism of chain growth and the way in which subunits are assembled. However, our work on the biosynthesis of O-antigen, a heteropolysaccharide component of Gram-negative bacteria, has shown that mechanism *B* (Fig. 1) is a realistic and likely alternative.

The authors are on the staff of the Massachusetts Institute of Technology, Cambridge, in the department of biology. Dr. Dankert is also a fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas, Republic of Argentina.

O-Antigen System—Structure and Synthesis of Lipopolysaccharide

All bacterial cells are bounded by a cytoplasmic membrane, and most have structures external to the membrane—for example, the rigid cell wall layer, the teichoic acids, and lipopolysaccharide. The function of the last component is not known, but there is reason to believe that lipopolysaccharide is involved in the interaction of the cell with the host animal. It is responsible for the endotoxin activity of Gram-negative enteric bacteria. Lipopolysaccharide, which is present on the cell surface, is a complex macromolecule having three main components—lipid A (*1*), the core, and the O-antigen (Fig. 2) (*2*, *3*). The core portion of the molecule is highly complex, containing glucose, galactose, *N*-acetylglucosamine, and several unusual components—a seven-carbon sugar, L-glycero-D-mannoheptose, an eight-carbon sugar acid, ketodeoxyoctonate (*4*), phosphate, and *O*-phosphorylethanolamine. The core is linked to lipid A, a unique structural lipid, by covalent linkage through ketodeoxyoctonate. The composition of the core is similar for all groups of Gram-negative enteric bacteria, in contrast to the structure of the O-antigen, which shows a high degree of variation and is group-specific. The cell synthesizes the lipid-A core structure and the O-antigen independently; then they are joined together in covalent linkage to form the complete lipopolysaccharide.

The pioneering discoveries by Nikaido (*5*), working with mutants of *Escherichia coli* and *Salmonella*, and the elegant studies by Osborn, Horecker, and their co-workers (*2*, *6*) demonstrated that in *S. typhimurium* the "core" is synthesized stepwise from the sugar nucleotide precursors UDP-glucose (*7*), UDP-galactose, and UDP-*N*-acetylglucosamine, and the appropriate lipopolysaccharide primers. At each step a different primer is required, in which the terminal nonreducing monosaccharide is the one to which the new sugar is being added. The core biosynthesis thus provides a good example of the *A* alternative proposed above (Fig. 1).

The biosynthesis of the O-antigen follows an entirely different pattern. In this we have evidence that the sugars are not transferred directly to the growing polymer but, rather, are assembled into an oligosaccharide precursor (*8*). In the case of the *Salmonella newington* O-antigen, which has a mannosyl-rhamnosyl-galactosyl repeating sequence (*9*) (Fig. 3) and is synthesized from the sugar nucleotide precursors UDP-galactose, TDP-rhamnose, and GDP-mannose (*10*), we have proposed the reaction scheme shown in Fig. 4 (*11*). In this scheme, to the classical requirements of activated sugar donors and polysaccharide primer a new factor is added, the antigen-carrier lipid phosphate (ACL phosphate). The role of ACL phosphate is to allow assembly of the trisaccharide repeating units prior to their polymerization into long chains, its lipophilic properties being suited to the hydrophobic nature of the membrane.

The first step in the proposed series of reactions is the transfer of galactose-1-phosphate from UDP-galactose to ACL phosphate to form galactose-P-P-ACL. The second step is the transfer of rhamnose from TDP-rhamnose to the galactosyl moiety to form rhamnosyl-galactosyl-P-P-ACL. The sequence is completed by the transfer of mannose to form mannosyl-rhamnosyl-galactosyl-P-P-ACL. The O-antigen is formed

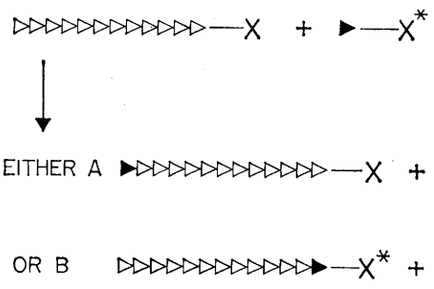

Fig. 1 (left). Schematic representation of the two possible general mechanisms of growth of linear biological polymers. Triangles, polymer subunits; × and ×*, activating groups.

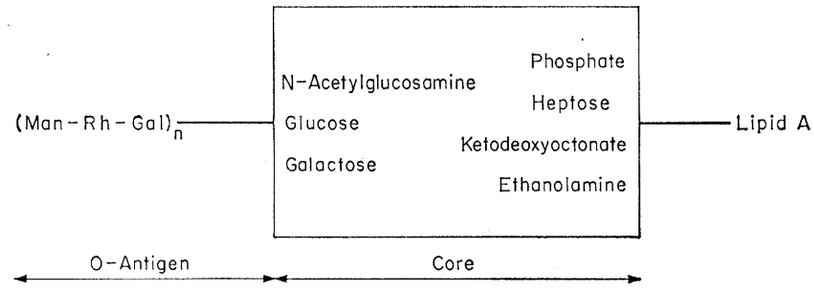

Fig. 2 (right). Structural components of lipopolysaccharide.

by the polymerization of the lipid-linked trisaccharide units, and is finally transferred to the core to produce the end product—that is, lipopolysaccharide.

In this scheme several points deserve discussion. First, as mentioned above, the presence of ACL introduces an intermediate stage in the building of the heteropolysaccharide: the sugars are not transferred directly to the growing chains but are transferred to form ACL-linked intermediates which become the direct precursors of the polysaccharide. Second, in the first step of the cycle, galactose is transferred, together with a phosphate group, to a phosphate acceptor, the pyrophosphate bridge originally present in the nucleotide donor being restored in this way. From an energetic point of view, the intermediates are thus kept at an activated level in order to allow their subsequent polymerization. Third, the ACL pyrophosphate, formed after the transfer of trisaccharide to the growing chain, loses a phosphate group in order to reenter the cycle. This hydrolytic reaction leading to the release of inorganic phosphate could act as an additional driving force in the direction of synthesis, or could be the consequence of a more complex mechanism, as suggested below.

Fourth, ACL phosphate provides a unique example of a functional phospholipid. From the beginning it was clear that ACL was not related to the major phospholipids present in *Salmonella*, and recent studies (*12*) have led us to propose the structure shown in Fig. 5. The basic component is a polyisoprenoid alcohol consisting of 11 isoprene units. Very probably it is directly linked to the sugars through the pyrophosphate bridge, but more detailed studies are necessary to unambiguously establish the complete structure. A structurally related lipid is involved in the biosynthesis of the bacterial cell wall mucopeptide (*13*), as discussed below. Similar polyprenols have been described in several bacterial species (*14*), molds (*15*), mammalian tissues (*16*), and higher plants (*17*). Whether or not they are related to complex polysaccharide biosynthesis is a point that deserves attention.

Finally, the polymerization of the repeating units offers the two alternatives mentioned above. These are discussed in detail in the next section.

Experiments on Direction of O-Antigen Chain Growth

In order to determine the direction of chain growth in O-antigen biosynthesis it is necessary only to determine to which end of the nascent chain new subunits are added. When we equate the triangles of Fig. 1 to the mannosyl-rhamnosyl-galactosyl repeating unit, and X and X* to the polyisoprene pyrophosphate discussed above (Fig. 5), it may be seen that the possible mechanisms differ in type of growth: in one case it is the activated end (carrying ACL pyrophosphate) which receives each new trisaccharide; in the other case it is the nonactivated end. Since the activated end of a growing antigen chain may be readily distinguished chemically as being the only sugar residue with an easily released reducing group, decision between the two types of growth rests on the question, Are new trisaccharide units added to the reducing end of the chain? If the answer is yes, then *B* of Fig. 1 is probably the operative mechanism.

Experiments were carried out to determine the direction of chain growth, based on the addition of radioactively labeled precursor to a system in which antigen chains were being synthesized, and, after a short period, extraction of the intact chains to see how much radioactivity there was in the sugar at the activated end (*18*). The antigen chains were separated from the bulk of cellular proteins by means of treatment with hot phenol (*19*), which will also split the labile linkage between the sugar and its carrier. Since some of the antigen chains released in this way probably have a terminal phosphate group, the preparation was digested with the enzyme alkaline phosphatase. The terminal galactose residues were then reduced to galactitol with sodium borohydride. Further purification steps included dialysis and gel filtration to remove low-molecular-weight components. Compounds which, unlike the antigen chains, are charged at neutral *p*H were removed on an ion-

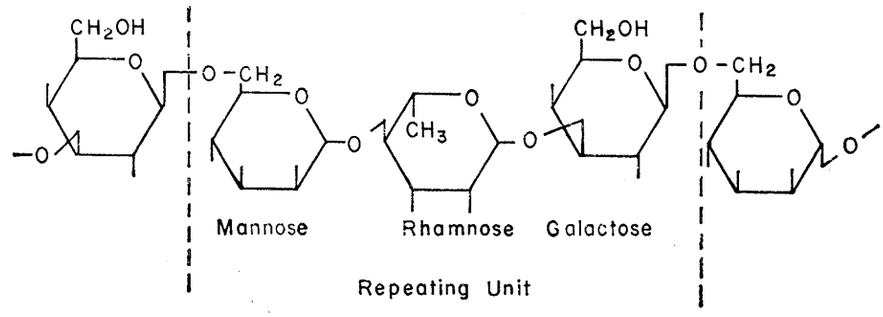

Fig. 3. Basic repeating sequence of the *Salmonella newington* O-antigen.

exchange resin. Finally the antigen chains were hydrolyzed to give a mixture of the monosaccharides: mannose, rhamnose, galactitol, and galactose. Galactitol was separated from this mixture by standard chromatographic procedures, and its radioactivity measured.

In order to have controls with which to compare the amount of radioactive galactitol recovered, parallel experiments were carried out in which the short exposure to radioactivity was followed by a comparatively long period of exposure to nonradioactive precursor (the chase). Subsequent purification of the antigen and isolation of the galactitol were carried out as before. Since the effect of the chase should be to bury the radioactive growing ends of the antigen chains, there should be a pronounced fall in the C^{14}-galactitol content if growth is taking place at the reducing end. If the chains are growing in the other direction, then only comparatively few chains, which are initiated during the pulse, should have radioactive reducing ends, and these will not be lost during the chase. This is shown diagrammatically in Fig. 6.

The first experiment involved the labeling of antigen chains, which were being synthesized in vivo, by the addition of C^{14}-glucose to cells growing in nutrient broth. Under these conditions the radioactivity is efficiently incorporated into O-antigen. In both this experiment and the one described next, a mutant strain of *Salmonella anatum* was used which is defective with respect to the terminal reaction of O-antigen biosynthesis (Fig. 4), that of addition of completed chains to the lipopolysaccharide core. In this way large amounts of antigen unattached to the core could be obtained.

Table 1. In vivo pulse experiment: recovery of galactitol from reduced O-antigen chains. Radioactive antigen was prepared from growing cultures pulse-labeled with C^{14}-glucose for 30 seconds, then grown in excess nonradioactive glucose for the periods indicated. Equivalent amounts of antigen were reduced with sodium borohydride-H^3 and subsequently hydrolyzed to yield monosaccharides. The galactitol fractions were isolated by standard chromatographic techniques.

Period of chase (min)	Radioactivity (count/min) in galactitol		C^{14}/H^3
	C^{14}	H^3	
0	830	630	1.3
5	29	690	0.04
15	38	1332	.03

Highly radioactive C^{14}-glucose was added to three growing cultures of the mutant strain, and after 60 seconds the growth of one was stopped. At this time a large excess of nonradioactive glucose (the chase) was added to the two remaining cultures, and growth was allowed to continue for 5 minutes in one and for 15 minutes in the other. Antigen chains were then extracted from both cultures, reduced, and analyzed as described. In order to correct for possible losses in extraction, tritiated sodium borohydride was used in the reduction, and the final results were expressed as the ratio of C^{14} to H^3 in galactitol.

Table 1 shows the amounts of C^{14} and H^3 recovered in the galactitol samples from this experiment. The results clearly show that only one sample—that in which the culture was labeled with C^{14}-glucose without a subsequent period of synthesis of nonradioactive antigen—has an appreciable amount of C^{14}-galactitol. This is exactly what one would expect if growth were occurring at the reducing end. Since all of the chains, whether or not they were labeled with C^{14}-glucose, were reduced with tritiated sodium borohydride, the ratio of C^{14} to H^3 is an intensive function, independent of individual differences in recovery. Table 1 shows that this value is over 30 times as high in the pulse sample as in the two chase samples.

The second experiment was carried out on antigen chains being synthesized in cell-free incubations. The cell preparations used have been described elsewhere (*9*) and synthesize normal O-antigen from UDP-galactose, GDP-mannose, and TDP-rhamnose. For the pulse, the incubation was with C^{14}-UDP-galactose for 2 minutes. For the chase, the incubation was continued for an additional 20 minutes in the presence of unlabeled UDP-galactose. The antigen was extracted from the incubation mixtures as before and then reduced, this time with nonradioactive sodium borohydride.

Since, following hydrolysis, all of the radioactivity was present as either galactose or galactitol, it was possible to chromatograph the monosaccharides directly. Figure 7 shows the result. In the pulse sample, the only radioactivity present coincides with galactitol, while, in the chase, the major component coincides with galactose,

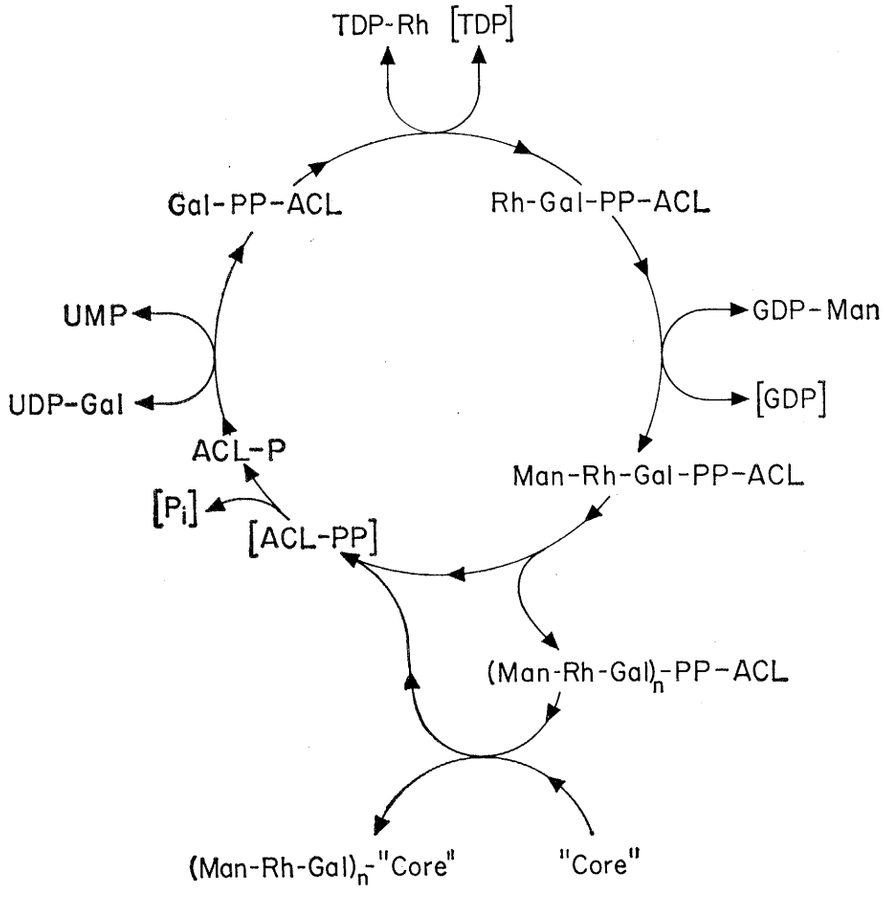

Fig. 4. Scheme for the biosynthesis of the *Salmonella* O-antigen.

only a small amount coinciding with galactitol. Clearly this is evidence that the chains were growing at the reducing end.

Taken together these two experiments strongly suggest that, in the biosynthesis of the O-antigen, polymerization proceeds by the addition of nascent chains to the subunits. In this respect it is more like the assembly of a protein or fatty acid than of glycogen. Growth from the reducing end provides a way in which the inert nonreducing end can be extended away from the enzyme system as growth proceeds. With this mechanism both the antigen polymerase which builds the chains and the translocase system which transfers them to the core act on the activated reducing end. In fact our result suggests that these two enzymes might be closely associated physically.

A specific formulation of the O-antigen synthesis mechanism is shown in Fig. 8. This shows the transfer of a hexasaccharide unit to trisaccharide-PP-ACL and the consequent formation of ACL pyrophosphate. Although detailed speculation on the mechanism is unwarranted at present, one important question should be discussed here.

Is the cleavage of ACL pyrophosphate coupled in some way with the polymerase reaction, or does ACL pyrophosphate undergo hydrolysis to ACL phosphate and to inorganic phosphate in a subsequent step following dissociation from the active site of the polymerase? If hydrolysis occurs during the course of the polymerization step, the mechanism of the reaction is undoubtedly more complex than the type of transglycosylation involved in the glycogen synthetase reaction. The free-energy change associated with the hydrolysis of the pyrophosphate linkage could, in fact, be coupled with conformational changes in enzyme structure or could be associated with other complex properties of the polymerase reaction mechanism per se.

If, on the other hand, ACL pyrophosphate is liberated from the active site of the polymerase, then the subsequent hydrolysis to ACL phosphate and to inorganic phosphate can be considered a means of regenerating ACL phosphate and a thermodynamic "driving force" for the overall cycle in the general sense.

The biosynthesis of the peptidoglycan component of the bacterial cell wall, which closely parallels the biosynthesis of the O-antigen, has been studied in great detail in several laboratories (*20–22*). The peptidoglycan is a polymer which has the basic repeating sequence *N*-acetylmuramyl-*N*-acetylglucosamine, with peptide substituents on the *N*-acetylmuramyl unit. This repeating sequence is formed on a lipid, prior to its polymerization, from the precursors UDP-*N*-acetylmuramyl-peptide and UDP-*N*-acetylglucosamine (*21*); the lipid is structurally related to the antigen carrier lipid, and the repeating sequence is linked to it through a pyrophosphate bridge (*13*).

The almost identical methods of assembly of the peptidoglycan and the O-antigen, and the fact that both are formed by membrane-bound enzymes, suggest that chain elongation might proceed by the same mechanism in the two systems. It is possible that in both systems lipid-linked nascent chains are transferred to the nonreducing end of each new lipid-linked subunit.

Review of Polysaccharide Synthesizing Systems

Let us turn our attention now to the question of the direction of chain growth of other polysaccharides. Table 2 contains a list of representative enzyme systems that have been studied in vitro. Beginning with glycogen and starch, it is clear that in both cases glucosyl residues are added to the nonreducing end groups of growing chains. Regardless of whether the rare reducing end groups in glycogen and starch molecules are free or bound, the structure of the product and the specificity of glycogen synthetase make it certain that addition takes place as shown in Fig. 9. Thus, when radioactive glucose units are added to glycogen chains from UDP-glucose-C^{14}, subsequent digestion with enzymes that attack nonreducing end groups leads to rapid release of the label (*23*). In the xylo-

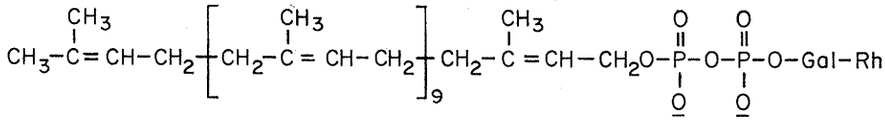

Fig. 5. Proposed basic structure of the disaccharide pyrophosphate antigen carrier lipid.

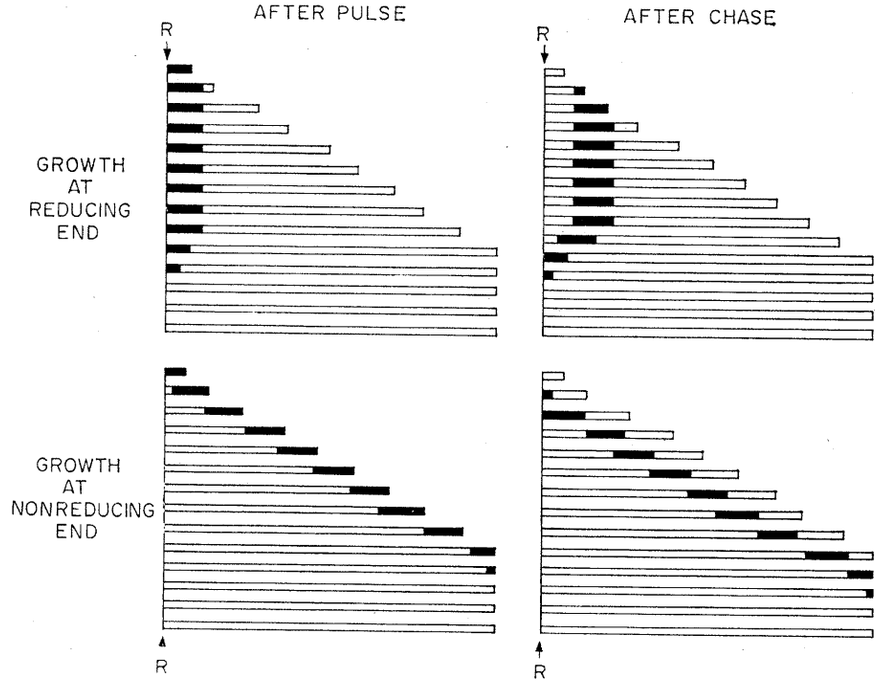

Fig. 6. The labeling patterns expected in growing antigen chains after a short period of exposure to radioactive precursor (pulse), or to a pulse followed by a period of exposure to nonradioactive precursor (chase). The chains are represented with their reducing ends (*R*) to the left, and the radioactive regions within them are shown as solid areas. The two possible directions of chain growth give different labeling patterns and may be distinguished by measurement of the amounts of radioactivity in the reducing ends.

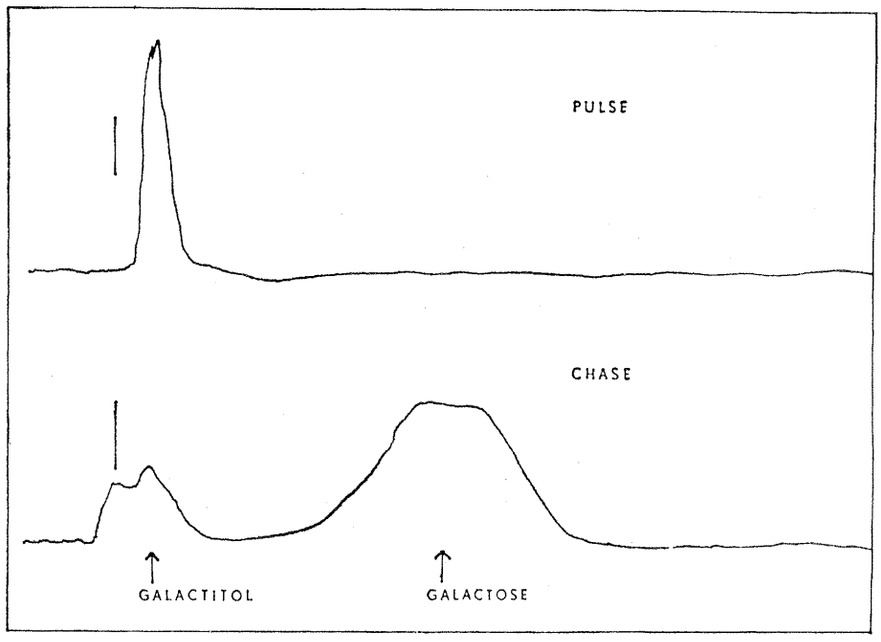

Fig. 7. Chromatography of the products of the in vitro experiment. Antigen containing C^{14}-galactose was synthesized in vitro in incubations containing C^{14}-UDP-galactose. In the top record the reaction was stopped after 2 minutes; in the bottom record, excess nonradioactive UDP-galactose was added after 2 minutes and the incubation was continued for 20 minutes. Both antigen preparations were purified, reduced with (nonradioactive) sodium borohydride, and hydrolyzed. The monosaccharides released were chromatographed directly.

dextrin xylotransferase system described by Feingold (*24*) it is also clear that addition takes place at the nonreducing ends of chains, since purified unactivated xylodextrins serve as acceptors.

For instance, in the reaction

$$\text{UPD-xyl}^* + \text{xyl-xyl}^+ \rightarrow \text{UDP} + \text{xyl}^*\text{—xyl—xyl}^+$$

the reducing group of the trisaccharide (xyl+) cannot add to UDP-xyl* because the reaction would be energetically unfavorable. Furthermore, any postulated transglycosylation reactions other than direct transfer from UDP-xyl* would lead to elimination of one or more of the xylose units. Experimentally it has been shown that trisaccharide primer gives rise to a tetrasaccharide product. Similar arguments apply to the chondroitin sulfate synthesizing system studied by Telser, Robinson, and Dorfman (*25*). These workers showed that an extract of embryonic epiphyses will catalyze the addition of *N*-acetylgalactosamine to oligosaccharides, with nonreducing terminal uronic acid residues, and the addition of glucuronic acid to oligosaccharides with nonreducing terminal *N*-acetylgalactosamine residues. Since the precursors and products are of known structure and chondroitin sulfate contains an alternating glucuronic acid, *N*-acetylgalactosamine sequence, there can be no doubt that addition takes place at the nonreducing terminal position.

As mentioned above, the lipopolysaccharide glucosyl and galactosyl transferase enzymes described by Rothfield, Osborn, and their co-workers also catalyze the addition of glycosyl residues to nonreducing positions in the lipopolysaccharide core. This has been shown by chemical and enzymatic analysis of the products of in vitro synthesis.

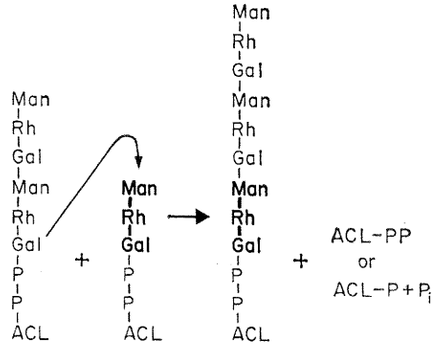

Fig. 8. Mechanism of chain elongation in the *Salmonella* O-antigen. The growing chain, represented by a hexasaccharide unit, is transferred to the lipid-linked trisaccharide repeating sequence. The sugar residues from the trisaccharide unit, which are shown in bold-face type, are at the reducing end of the resulting nonasaccharide unit.

The formation of nucleoside diphosphate oligosaccharides with more than two monosaccharide components can occur by either of the proposed mechanisms of Fig. 1. Thus the UDP derivative of *N*-acetylneuraminylgalactosyl-*N*-acetylglucosamine which has been isolated from colostrum (*26*) could be formed by sequential addition of galactose and *N*-acetylneuraminic acid from their active nucleotide derivatives to UDP-*N*-acetylglucosamine, or it could be formed by transfer of *N*-acetylneuraminylgalactose from the nucleotide diphosphate disaccharide to UDP-*N*-acetyl glucosamine. The same arguments apply to the formation of the UDP derivative of fucosylgalactosyl-*N*-acetylglucosamine, which has been isolated from milk (*27*). However, since the UDP derivative of galactosyl-*N*-acetylglucosamine has been isolated from both colostrum and milk, it seems most likely that mechanism *A* (Fig. 1) is operative in these cases.

In the other systems listed in Table 2 the direction of chain growth is not certain. As an illustration of the problems of interpretation let us consider the synthesis of hyaluronic acid (Fig. 10). When UDP-glucuronic acid and UDP-*N*-acetylglucosamine are incubated with a particulate fraction from group-A *Streptococcus* cells (*28*) or animal cells (*29*), sugar residues are incorporated into long chains of hyaluronic acid. A number of possible mechanisms for chain growth exist. For example, it is possible that glucuronic acid and *N*-acetylglucosamine are added sequentially to the nonreducing ends of growing chains by two separate enzymes or even by a single enzyme with two binding sites, as suggested originally by Markovitz and Dorfman (*28*). Addition of disaccharide to the nonreducing end group, with a hypothetical uridine diphosphate disaccharide as donor, has also been considered, but lack of evidence for the disaccharide nucleotide has made this possible mechanism seem less likely.

A parallel series of reaction schemes may be postulated for growth of hyaluronic acid at the reducing end of growing chains. It is only necessary to postulate the transfer of the reducing end of the growing chain to the nucleotide monosaccharide or disaccharide precursor at each step. The predictions to be made for these alternate mechanisms would be (i) that

Table 2. Representative polysaccharide-synthesizing systems.

Polymer	Polymer repeating unit	Polymer precursor	Type of growth	Reference
Glycogen	α-D-glucose (1→4)	UDP-D-glucose	Nonreducing	(*23*)
Starch	α-D-glucose (1→4)	UDP-D-glucose ADP-D-glucose	Nonreducing	(*30*)
Xylodextrin	β-D-xylose (1→4)	UDP-D-xylose	Nonreducing	(*24*)
Lipopolysaccharide core	[glucose, galactose, *N*-acetylglucosamine]	UDP-D-glucose UDP-*N*-acetylglucosamine UDP-D-galactose	Nonreducing	(*2, 6*)
Cellulose	β-D-glucose (1→4)	GDP-D-glucose	?	(*31*)
β-1, 3-glucan	β-D-glucose (1→3)	UDP-D-glucose	?	(*32*)
Mannan	D-mannose	GDP-D-mannose	?	(*33*)
Colominic acid	Sialic acid	CMP-sialic acid	?	(*34*)
Hyaluronic acid	β-D-glucuronic acid (1→3) β-*N*-acetylglucosamine (1→4)	UDP-D-glucuronic acid UDP-*N*-acetylglucosamine	?	(*28, 29*)
Teichuronic acid	Glucuronic acid (1→3?) *N*-acetylglucosamine	UDP-D-glucuronic acid UDP-*N*-acetylgalactosamine	?	(*35*)
Chondroitin sulfate	β-D-glucuronic acid (1→3) β-*N*-acetylgalactosamine (1→4)	UDP-D-glucuronic acid UDP-*N*-acetylgalactosamine	Nonreducing	(*25*)
Pneumococcal polysaccharides:				
Type III	β-D-glucuronic acid (1→4) β-D-glucose (1→3)	UDP-D-glucuronic acid UDP-D-glucose	?	(*36*)
Type VIII	D-galactose (1→4) β-D-glucuronic acid (1→4) β-D-glucose (1→4) D-galactose	UDP-D-glucose UDP-D-galactose UDP-D-glucuronic acid	?	(*37*)
Peptidoglycan	β-D-*N*-acetyl-D-glucosamine (1→4) β-D-*N*-acetylmucopeptide (1→4)	UDP-*N*-acetylmucopeptides UDP-*N*-acetylglucosamine	?	(*20*)
Teichoic acids:				
Glycerol	Glycerol phosphate	CDP-glycerol	?	(*38*)
Ribitol	Ribitol phosphate	CDP-ribitol	?	(*38*)
Aminosugar-glycerol	*N*-acetylglucosamine 1-phosphate-glycerol phosphate	UDP-*N*-acetylglucosamine CDP-glycerol	?	(*39*)

growing chains carry a UDP residue at the reducing end, and (ii) that pulse experiments would lead to rapid labeling of the reducing end of chains. Both of these predictions can be tested experimentally.

If a specific carrier other than UDP participates in the reaction cycle, then additional enzymes would be involved in the transfer from the nucleotide sugar derivative to this carrier. In any case, it is unlikely that the hyaluronic acid mechanism is exactly parallel to O-antigen synthesis, since both nucleotide sugars yield UDP following transfer in the hyaluronic acid system. While the hydrolysis of lipid pyrophosphate to lipid monophosphate obviously aids in driving the O-antigen cycle in the direction of synthesis, no comparable step can exist in hyaluronic acid synthesis. The most nearly analogous mechanism would be as follows:

$$\text{UDP-X} + \text{L-PP} \rightleftarrows \text{UDP} + \text{L-PP-X}$$

$$\text{L-PP-X} + \text{UDP-Y} \rightarrow \text{UDP} + \text{L-PP-XY}$$

$$n\,[\text{L-PP-XY}] - \text{L-PP-(XY)}_n + n - 1\,[\text{L-PP}].$$

Here X and Y = *N*-acetylglucosamine and glucuronic acid and L = carrier. Although the lack of hydrolysis of the pyrophosphate makes this scheme less attractive, it by no means rules it out. The overall equilibrium for O-antigen synthesis or hyaluronic acid synthesis from the nucleotide sugar precursor will probably be favorable even without the additional energy provided by the hydrolysis of lipid pyrophosphate.

A number of alternative reactions could be formulated along similar lines. The question is whether or not the reducing end groups of growing chains are in an activated state and partici-

Fig. 9. The transfer of a glucose unit (*G*) from UDP-glucose to acceptor glycogen by glycogen synthetase. The glucose added is distinguished by an asterisk (*G**) and is transferred to a nonreducing end of the glycogen molecule.

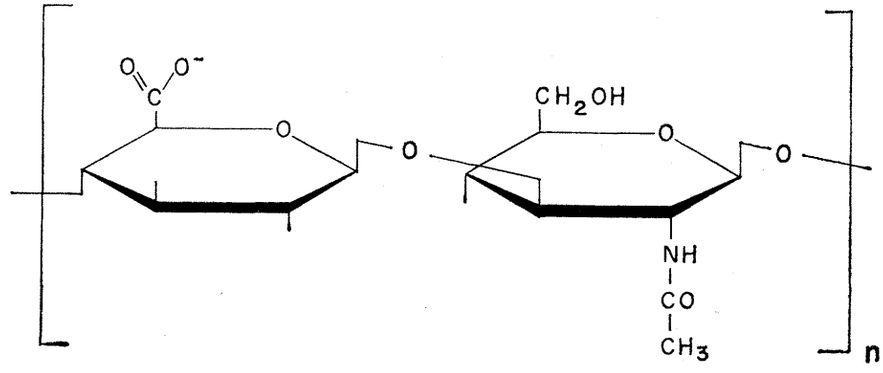

Fig. 10. Repeating sequence of hyaluronic acid.

pate in transfer reactions. These arguments and mechanisms could be extended in the discussion of the other systems listed in Table 2. We feel, however, that no purpose would be served by further speculation in the absence of experimental data. Here we wish only to point out the open questions and possible areas for future research.

Summary

The biosynthesis of a bacterial polysaccharide—the surface O-antigen of *Salmonella newington*—differs in several respects from the more classical example of glycogen synthesis. Sugars are not transferred directly to the antigen from sugar nucleotide precursors but are transferred first into lipid-linked oligosaccharides. Growth of the polysaccharide chain then occurs by assembly of these lipid-linked precursors at the reducing end of the polymer rather than at its nonreducing end as in glycogen. This method of assembly, in which nascent chains are transferred to the next subunit, is analogous to the growth of proteins or fatty acids. It seems possible that these differences reflect the more complex requirements of a surface polysaccharide synthesized by membrane-bound enzymes. If this is the case, then several other polysaccharide systems may be synthesized by comparable mechanisms.

References and Notes

1. O. Westphal, A. Nowotny, O. Lüderitz, H. Hurni, E. Eichenberger, *Pharm. Acta Helv.* **33**, 401 (1958).
2. M. J. Osborn, S. M. Rosen, L. Rothfield, L. D. Zeleznick, B. L. Horecker, *Science* **145**, 783 (1964).
3. O. Lüderitz, A. M. Staub, O. Westphal, *Bacterial. Rev.* **30**, 192 (1966).
4. E. C. Heath and M. A. Ghalambor, *Biochem. Biophys. Res. Commun.* **10**, 340 (1963).
5. H. Nikaido, *Biochim. Biophys. Acta* **48**, 460 (1961); ———, in *The Specificity of Cell Surfaces*, B. D. Davis and L. Warren, Eds. (Prentice-Hall, Englewood Cliffs, N.J., 1967); ———, Y. Naide, P. H. Mäkelä, *Ann. N.Y. Acad. Sci.* **133**, 299 (1966).
6. M. J. Osborn, S. M. Rosen, L. Rothfield, B. L. Horecker, *Proc. Nat. Acad. Sci. U.S.* **48**, 1831 (1962); M. J. Osborn and L. D'Ari, *Biochem. Biophys. Res. Commun.* **16**, 568 (1964); S. M. Rosen, M. J. Osborn, B. L. Horecker, *J. Biol. Chem.* **239**, 3196 (1964); L. Rothfield, M. J. Osborn, B. L. Horecker, *ibid.*, p. 2788; M. J. Osborn, *Ann. N.Y. Acad. Sci.* **133**, 375 (1966).
7. Abbreviations used in this article are as follows: ACL, antigen carrier lipid; ADP-glucose, adenosine diphosphate-D-glucose; CDP, cytidine disphosphate; CMP, cytidine monophosphate; Gal, D-galactose; GDP, guanosine diphosphate; M or Man, D-mannose; P, phosphate; Pi, inorganic phosphate; PP, pyrophosphate; Rh, L-rhamnose; TDP, thymidine diphosphate; TDP-rhamnose, thymidine diphosphate-L-rhamnose; UDP, uridine diphosphate; UDPG, uridine diphosphate D-glucose; UMP, uridine-5′-monophosphate; xyl, D-xylose.
8. I. M. Weiner, T. Higuchi, L. Rothfield, M. Saltmarsh-Andrew, M. J. Osborn, B. L. Horecker, *Proc. Nat. Acad. Sci. U.S.* **54**, 228 (1965); I. M. Weiner, T. Higuchi, M. J. Osborn, B. L. Horecker, *Ann. N.Y. Acad. Sci.* **133**, 391 (1966); M. J. Osborn and I. M. Weiner, *Federation Proc.* **26**, 70 (1967); P. W. Robbins, A. Wright, M. Dankert, *J. Gen. Physiol.* **49**, 331 (1966).
9. P. W. Robbins and T. Uchida, *Biochemistry* **1**, 323 (1962); *J. Biol. Chem.* **240**, 375 (1965).
10. P. W. Robbins, A. Wright, J. L. Bellows, *Proc. Nat. Acad. Sci. U.S.* **52**, 1302 (1964).
11. A. Wright, M. Dankert, P. W. Robbins, *ibid.* **54**, 235 (1965); M. Dankert, A. Wright, W. Kelley, P. W. Robbins, *Arch. Biochem. Biophys.* **116**, 425 (1966).
12. A. Wright, M. Dankert, P. Fennessey, P. W. Robbins, *Proc. Nat. Acad. Sci. U.S.*, **57**, 1798 (1967).
13. Y. Higashi, J. L. Strominger, C. C. Sweeley, *ibid.*, p. 1878.
14. K. J. I. Thorne and E. Kodicek, *Biochem. J.* **99**, 123 (1966); C. M. Allen, W. Alworth, A. Macrae, K. Bloch, *J. Biol. Chem.* **242**, 1895 (1967).
15. K. J. Stone, P. H. W. Butterworth, F. W. Hemming, *Biochem. J.* **102**, 443 (1967).
16. J. Burgos, F. W. Hemming, J. F. Pennock, R. A. Morton, *ibid.* **88**, 470 (1963).
17. A. R. Wellburn, J. Stevenson, F. W. Hemming, J. F. Pennock, *ibid.*, p. 325. K. J. Stone, A. R. Wellburn, F. W. Hemming, J. F. Pennock, *ibid.*, p. 325.
18. Complete details of these experiments are given by D. Bray and P. W. Robbins, *Biochem. Biophys. Res. Commun.* **28**, 334 (1967).
19. O. Westphal, O. Lüderitz, F. Bister, *Z. Naturforsch*, **7b**, 148 (1952).
20. A. N. Chatterjee and J. T. Park, *Proc. Nat. Acad. Sci. U.S.* **51**, 9 (1964); P. M. Meadow, J. S. Anderson, J. L. Strominger, *Biochem. Biophys. Res. Commun.* **14**, 382 (1964).
21. J. S. Anderson, M. Matsuhashi, M. A. Haskin, J. L. Strominger, *Proc. Nat. Acad. Sci. U.S.* **53**, 881 (1965).
22. W. G. Struve and F. C. Neuhaus, *Biochem. Biophys. Res. Commun.* **18**, 6 (1965); J. L. Strominger, K. Izaki, M. Matsuhashi, D. J. Tipper, *Federation Proc.* **26**, 9 (1967).
23. L. F. Leloir, J. M. Olavarria, S. H. Goldemberg, H. Carminatti, *Arch. Biochem. Biophys.* **81**, 508 (1959).
24. D. S. Feingold, E. F. Neufeld, W. Z. Hassid, *J. Biol. Chem.* **234**, 488 (1959).
25. A. Telser, H. C. Robinson, A. Dorfman, *Arch. Biochem. Biophys.* **116**, 458 (1966).
26. G. W. Jourdian, F. Shimizu, S. Roseman, *Federation Proc.* **20**, 161 (1961).
27. A. Kobata, *Biochem. Biophys. Res. Commun.* **7**, 346 (1962).
28. A. Markovitz and A. Dorfman, *J. Biol. Chem.* **237**, 273 (1962).
29. L. Glaser and D. H. Brown, *Proc. Nat. Acad. Sci. U.S.* **41**, 253 (1955); N. Ishimoto, H. M. Temin, J. L. Strominger, *J. Biol. Chem.* **241**, 2052 (1966).
30. L. F. Leloir, M. A. R. de Fekete, C. E. Cardini, *J. Biol. Chem.* **236**, 636 (1961).
31. A. D. Elbein, G. A. Barber, W Z. Hassid, *J. Amer. Chem. Soc.* **86**, 309 (1964).
32. L. R. Marechal and S. H. Goldemberg, *J. Biol. Chem.* **239**, 3163 (1964); D. S. Feingold, in *Methods in Enzymology*, E. F. Neufeld and V. Ginsburg, Eds. (Academic Press, New York, 1966), vol. 8, p. 404.
33. I. D. Alagranati, N. Behrens, H. Carminatti, E. Cabib, in *Methods in Enzymology*, E. F. Neufeld and V. Ginsburg, Eds. (Academic Press, New York, 1966), vol. 8, p. 411.
34. D. Aminoff and F. D. Kundig, *ibid.*, p. 419.
35. R. C. Hughes, *Biochem. J.* **101**, 692 (1966).
36. E. E. B. Smith and G. T. Mills, in *Methods in Enzymology*, E. F. Neufeld and V. Ginsburg, Eds. (Academic Press, New York, 1966), vol. 8, p. 446.
37. G. T. Mills and E. E. B. Smith, *Federation Proc.* **21**, 1089 (1962).
38. M. M. Burger and L. Glaser, *J. Biol. Chem.* **239**, 3168 (1964); L. Glaser, *ibid.*, p. 3178.
39. N. L. Blumson, L. J. Douglas, J. Baddiley, *Biochem. J.* **100**, 26c (1966).
40. The ideas and experiments presented in this article arose from a discussion between one of us (P.W.R.) and Dr. Fritz Lipmann. We would like to express our appreciation of Dr. Lipmann's continuing interest and thoughtfulness. His contribution to this problem is an example of his clear insight into the central questions of biochemistry and biology. This investigation was supported by U.S. Public Health Service research grant AM 06803-15 from the National Institute of Arthritis and Metabolic Diseases.