development of convective precipitation.

The big sagebrush is a particularly valuable plant for detecting change in the environment, especially an improved water economy. Precipitation increases at almost any time of the year influence the radial growth of this long-lived sensitive plant. The ring width of sagebrush from the control site has remained stable throughout the development of the irrigated area, emphasizing the improbability of large increases in precipitation due to nearby irrigation.

In several cases—for the minimum temperature at Waterville and Sunnyside, particularly, and for the rainfall records of Waterville and Ephrata—judgments based on the quality of a record or its constancy were made. Changes in the location and exposure of these sites defeat a rigorous analysis of climatic modification in their areas. Even with an increased number of stations now providing weather records, the variety of elements measured is poor. A number of volunteer weather stations initiated during the early, wetter part of the century, when dryland agriculture flourished, were lost during the widespread abandonment of farms in the dry years. Reestablishment of several of these measuring stations, for even several years, would assist in future investigations.

The question of climatic change due to man's activities is currently quite popular. Both planned and inadvertent modifications of the environment, for better or worse, have occurred and will continue to do so. We consider our generally negative results particularly of interest relative to the application of water to an area on a smaller but more intensive scale; for example, in disposition of sewage effluent on forest or dryland watersheds, power plant cooling, or warm water irrigation at the sites of future nuclear plants. Site changes understandably will occur, but the widespread climatic effects may well be minimal.

The sensitive nature of an ecosystem, including the behavior and distribution of plants and animals, offers a unique opportunity to document climatic changes that continue to occur (*11*). Along with improved weather records, an increasing emphasis on identification and protection of relic areas, further identification of sensitive plants through ring width chronology, and correlation of these plant responses to past and present climate would be valuable tools in research on arid lands.

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Polymorphism of the Somatic Antigen of Yeast

Yeast mannans exhibit species specific structures that are analogous to animal and bacterial antigens.

Clinton E. Ballou and William C. Raschke

A characteristic feature of bacterial and animal cell surfaces is the presence of a variety of specific somatic antigens, many of which involve the carbohydrate components of glycoproteins, glycolipids, lipopolysaccharides, and polysaccharide-protein complexes. Particularly well characterized are the lipopolysaccharide O antigens of enteric bacteria (*1*) and the blood group substances of animal cells (*2*). Although most readily detected by immunochemical methods, these carbohydrate-containing macromolecules may function as virus receptors and as sites for specific cell-cell interaction. They are species specific and may undergo transformation (*3*). It has been speculated that the numerous bacterial viruses, which infect enteric bacteria and bring about the transformation of their O antigens, provide the bacterium with a multitude of disguises that may assist it in surviving attack by the immune system of the animal host (*4*). Over 60 different antigens have been detected on the erythrocyte surface. It is generally thought, although there is little experimental support for the notion, that the many antigens of animal cell surfaces are involved in processes requiring contact informational exchange, such processes being presumed to occur in development, specific cellular adhesion, "homing," and self-recognition.

Specific surface antigens have not been shown to play an important role in yeast physiology except, possibly, in the sexual agglutination reaction (*5*). Nevertheless, yeasts do possess a similar system of such antigens and, as with animal and bacterial cells, the evidence for species specificity in the surface

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structure of yeasts first became apparent from immunological studies. Although the serological determinants of yeast cells have not been defined to the same extent as those of the enteric bacteria, which led to the Kauffmann-White classification of *Salmonella* (*6*), it has been clear for some time that there is considerable variety in the surface antigens of yeasts (*7*). In this article we discuss some recent studies on the immunochemistry of the yeast cell surface and show that the antigenic properties of yeasts can be explained mainly on the basis of the mannan-protein component of the cell wall. We also describe some studies in which we are attempting to define the mechanism of biosynthesis of yeast mannan and the genetic control of its structure.

Yeast Cell Wall Structure and Immunology

Common yeasts, such as bakers' yeast, elaborate a thick cell wall that is constructed primarily of three classes of macromolecules—glucan, a mannan-protein complex, and chitin. These make up 80 to 90 percent of the dry weight of the wall, with lipid accounting for the remainder. Although the precise arrangement of the components has not been established, the cell wall has been shown to be built up around a rigid glucan layer, the surface of which is coated with the mannan-protein that is interspersed with the glucan and penetrates the periplasmic space. Chitin is found primarily in the bud scars. A detailed description of the structure and properties of the yeast cell wall is provided by Phaff (*8*).

Investigations on the immunochemistry of yeasts, which until recently were relatively limited in scope, have been stimulated by a renewed interest in the serological detection and identification of yeast pathogens (*7, 9*). Hasenclever and Mitchell, who have done extensive immunological work on the genus *Candida*, were able to distinguish two antigenic groups within the species *Candida albicans*, group A possessing all of the determinants found on group B cells, plus one or more other determinants (*10*). Other studies on the cross-reactivities of yeasts have established that closely related species have many determinants in common, but it has also been found that identical or very similar antigenic determinants are present on the surfaces of yeasts in widely different taxonomic groups (*11*).

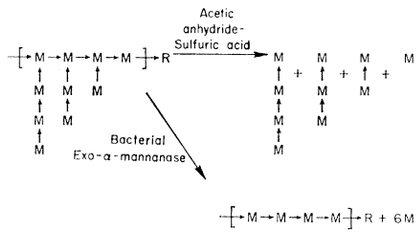


Fig. 1. Illustration of reactions employed for the selective degradation of the polysaccharide component of yeast mannan for the purpose of establishing the structure of the backbone and of the side chains. The acetolysis reaction is based on a preferential attack by the acetylium ion, $CH_3C{=}O^+$, on the 1→6 linkages. (*M*, D-mannopyranose; *R*, protein.)

Thus, attempts to classify yeasts on the basis of their serology have not been very successful, and the reason for this becomes apparent in our discussions of the immunochemistry of the yeast cell.

It was recognized almost 50 years ago that the antigenic material on the yeast cell surface was a polysaccharide (*12*). However, it was not until 1964 that identity between the antigen and the mannan component was established by the demonstration that purified mannan was able to inhibit completely the agglutination of whole cells by the homologous antiserum (*13*). Thus, since the principal antigen of the yeast cell is the mannan-protein component, the serological differences between yeasts must reflect differences in mannan structure. It is this problem of rationalizing the immunology of yeasts in terms of the chemistry of the cell wall mannan that has attracted our attention for several years.

The structure of yeast mannans. Chemical studies by Haworth *et al.*, as early as 1941 (*14*), showed that bakers' yeast mannan was composed almost entirely of D-mannose, that it was highly branched, and that it contained 1→2, 1→3, and 1→6 linkages between the sugar units. Data on the methylation of mannan indicate a number of possible structures, and in 1961 Peat *et al.* (*15*) attempted to distinguish among such structures by partial acid hydrolysis which gave small yields of 1→6-linked oligosaccharides. This suggested that the mannan possessed a 1→6-linked backbone with side chains of 1→2- and 1→3-linked mannose units. Other properties, such as the optical rotation, indicated that the sugars were linked mainly in the α-configuration.

During the last few years in this laboratory, two general procedures have been developed which make it possible to dissect the polysaccharide part of the mannan molecule in a controlled manner and obtain definitive evidence for its structure (Fig. 1). In one procedure, first applied to mannan by Gorin and Perlin (*16*), use is made of the acetolysis reaction that causes cleavage preferentially at 1→6 linkages by an acid-catalyzed process. Under appropriate conditions, acetolysis splits the 1→6 linkages and yields an assortment of fragments which can be separated readily by gel filtration (*17*).

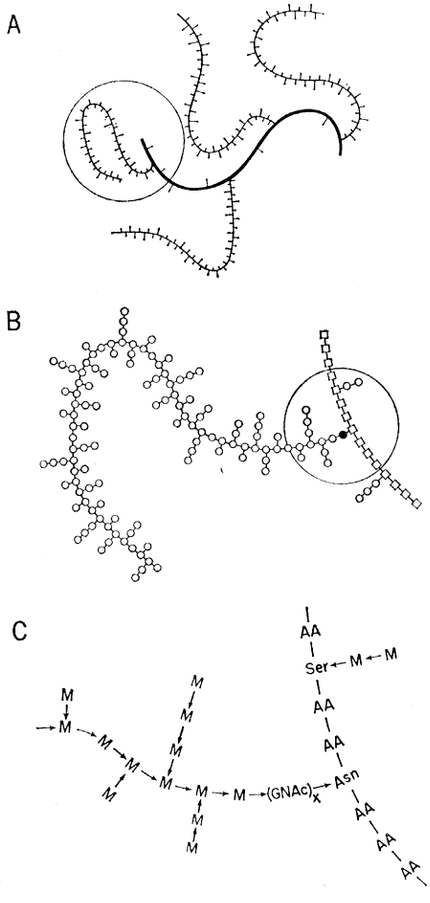


Fig. 2. Models illustrating structural features of a mannan-protein. (A) The heavy line is the protein portion, to which is attached four polymannose chains and several short oligosaccharide units. (B) An enlargement of the encircled portion in (A), illustrating the highly branched structure of the polysaccharide. (C) An enlargement of the encircled portion in (B), showing details of the attachment of carbohydrate to the protein via asparagine and serine. The linkages and the number of *N*-acetyl-D-glucosamine units at the point of attachment have not been established. By analogy with other glycoproteins, it is probable that there are two glucosamine units. (*AA*, amino acid; *Asn*, asparagine; *Ser*, serine; *GNAc*, *N*-acetyl-D-glucosamine; *M*, D-mannopyranose.)

Because many yeast mannans, as suggested earlier (*15*), possess a 1 → 6-linked backbone, these fragments represent the side chains of the mannan.

To determine the backbone structure of mannans, we used a soil bacterium that grows on mannan as the sole carbon source (*18*). This microorganism secretes an exo-α-mannanase that preferentially hydrolyzes the side chains from the mannan molecule and leaves the backbone core which can be isolated and characterized by classical methods (*19*). The action of this enzyme on bakers' yeast mannan yielded a linear 1 → 6-linked polymannose, about 50 units in length, that represented 35 percent of the original mannan molecule. This result agreed well with the methylation data (*14*) which indicated that 35 percent of the mannose units were 1 → 6-linked.

Employing these two procedures, in conjunction with methylation analysis by mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy, we have arrived at a generalized structure for the bakers' yeast mannan polysaccharide that consists of an α-1 → 6-linked backbone with side chains of various lengths containing α-1 → 2 and α-1 → 3 linkages. Work in other laboratories suggests that the linkage of mannan to protein involves both a direct attachment of short oligosaccharides to serine and threonine residues and linkage of larger polysaccharide units, by way of *N*-acetylglucosamine, to asparagine (*20*). Whether there is a specific order of the side chains in the polymer has not been determined; neither has the structure of the protein component in the mannan-protein complexes. A general model for bakers' yeast is depicted in Fig. 2, and Fig. 3 shows a space-filling model of the structure in Fig. 2C which emphasizes the brushlike shape of the mannan chain of this yeast.

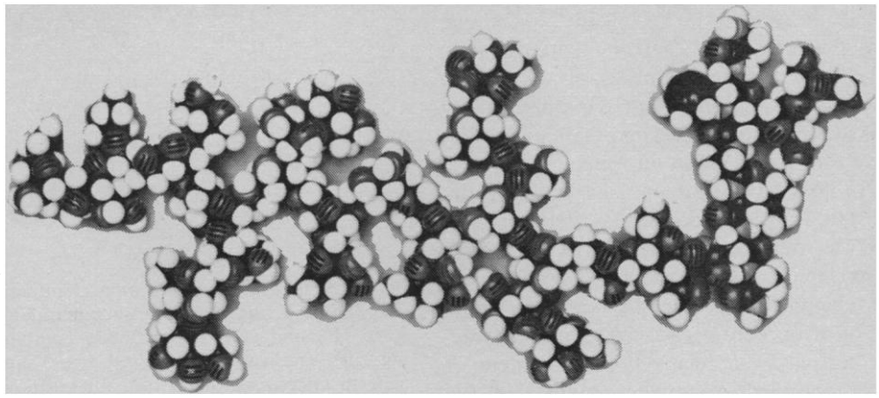


Fig. 3. A space-filling model of the structure shown in Fig. 2C, which reveals the brushlike nature of the mannan polymer. The peptide chain is on the right side.

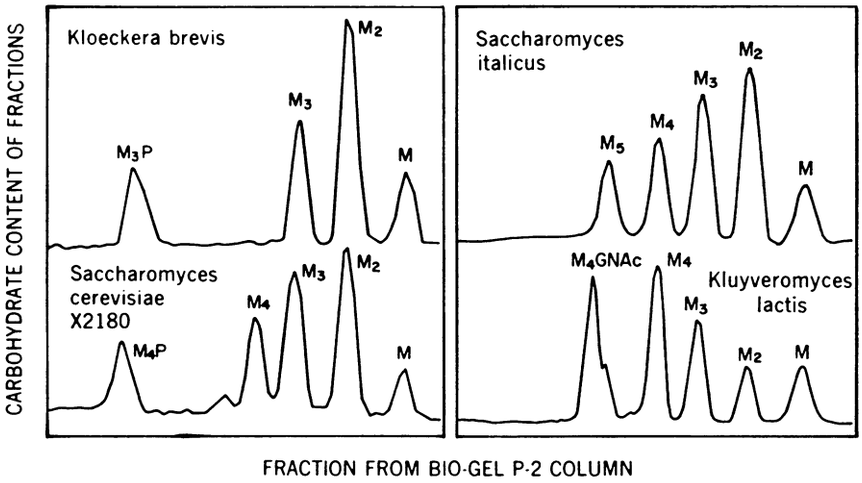


Fig. 4. Acetolysis patterns of four yeast mannans. Mono- to pentasaccharides are indicated by *M* to M_5, while M_3P is mannotriose phosphate. The phosphorylated fragments from the *S. cerevisiae* X2180 mannan consist of a small amount of M_3P and a larger amount of M_4P (*26*). The separations were done on a 200-cm Bio-Gel P-2 column with water as the eluent. M_1GNAc is the *N*-acetylglucosamine derivative of mannotetraose.

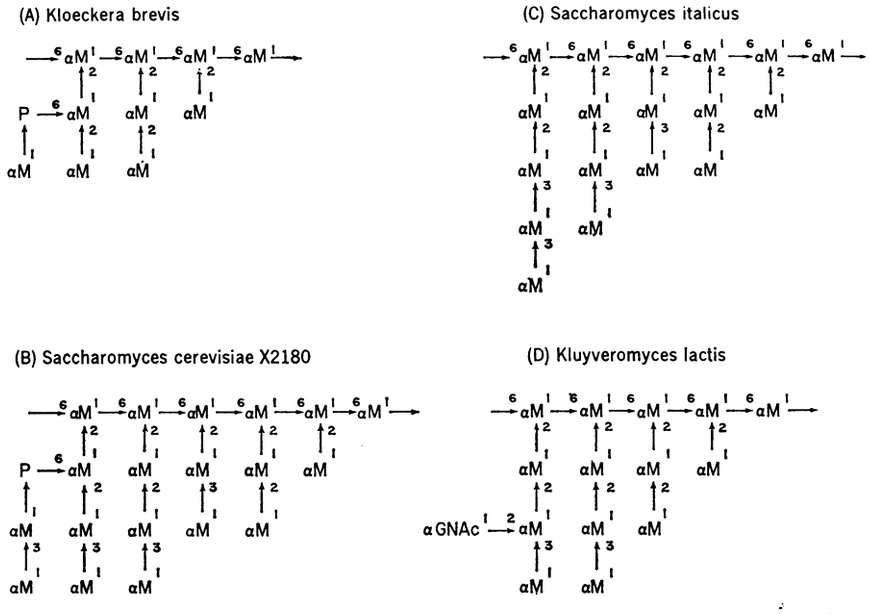


Fig. 5. Detailed structures of the four mannans for which acetolysis patterns are given in Fig. 4. The order of side chains along the backbone, if any, is not known.

Yeast Mannan Chemotypes

Although the acetolysis procedure proved valuable for structural analysis of yeast mannans, it also gave an early clue to the existence of species differences in mannan structure. Work in this laboratory (*17, 21, 22*) showed that different yeast mannans gave different assortments of side chain fragments in the acetolysis reaction (Fig. 4). While it is known that all yeast mannans do not have the relatively simple structure of bakers' yeast, and

that some yeast mannans do not have exclusively $1 \rightarrow 6$-linked backbone structures (*23-25*), the patterns produced by the acetolysis procedure are useful for comparing the polysaccharides and for preparing potential haptens from the mannan antigens.

We have characterized in some detail four polysaccharides that serve to illustrate the structural variety in closely related yeast mannans. The mannan chemotype in Fig. 5A was first found in *Kloeckera brevis* (*26*) and is characterized by a relatively high content of diesterified phosphate. Mild acid hydrolysis releases mannose, which is glycosidically linked to phosphate, while the second phosphate linkage is to position 6 of a side chain mannose unit. Acetolysis of *Kloeckera brevis* mannan yields mannose, mannobiose, mannotriose, and a phosphorylated side chain, M_3P (see Fig. 4). Methylation analysis of the side chains showed that only $1 \rightarrow 2$ linkages were present. Subsequently, mannan with a similar structure has been found in *Saccharomyces cerevisiae* brewing strains (*27*) and in *S. cerevisiae* 4484-24D and A364A (*28, 29*).

Saccharomyces cerevisiae S288C mannan yields mannose, mannobiose, mannotriose, and mannotetraose, with the linkages shown in Fig. 5B. Characteristic of this mannan is the mannotetraose side chain, which contains a terminal α-$1 \rightarrow 3$-linked mannose unit, and the α-$1 \rightarrow 3$-linked mannobiosyl unit attached in a phosphodiester linkage (*26, 30*). A similar mannan is elaborated by bakers' yeast (*17*) and *S. cerevisiae* X2180 (*25, 31*).

On acetolysis, *S. italicus* mannan yields a mannopentaose side chain (Fig. 4) in addition to the fragments found in *S. cerevisiae* S288C mannan. Structural analyses have demonstrated the presence of two $1 \rightarrow 3$ linkages which occur together at the nonreducing end of the pentasaccharide unit (*30*).

The structure of *Kluyveromyces lactis* mannan (shown in Fig. 5D) is also similar to that of *S. cerevisiae*, but it has one additional kind of side chain in which *N*-acetylglucosamine is attached in α-$1 \rightarrow 2$ linkage to the penultimate sugar of a mannotetraose unit (*32*). Both *Kluyv. marxianis* and *Kluyv. dobzhanski* make a similar mannan (*30*) (Fig. 5C).

Chemotype versus serotype. We are now in a position to describe differences in yeast mannans on the basis of two features. One is the chemotype, or the

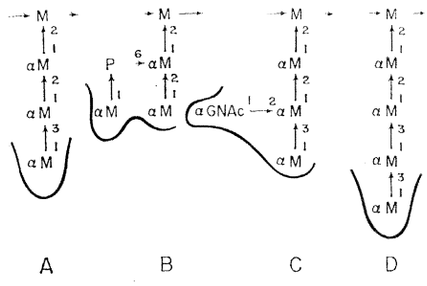


Fig. 6. Structures of the immunodominant side chains in the mannans shown in Fig. 5. In (B) and (C), the antibody combining sites (indicated by curved lines) appear to encompass parts of both terminal units. (Abbreviations as in Figs. 2 and 4.)

structure that is derived from characterization of the acetolysis products and the backbone obtained by exo-α-mannanase digestion. The other is the serotype, which relates the antigenic properties of the cell surface mannan to specific chemical structures. We have made these correlations utilizing standard procedures (*33*).

After preparation of rabbit antiserums against whole yeast cells, the precipitin reaction between antiserum and pure mannan can be analyzed for inhibition by the acetolysis fragments. Our most detailed study has been made with the four yeasts mentioned previously. The chemotypes of these yeast mannans are shown in Fig. 5, and the immunodominant structures that define the serotypes are indicated by the partial structures in Fig. 6 in which the heavy curved lines delineate the presumed antibody combining sites.

In the case of *S. cerevisiae* S288C and X2180, it is evident that the tetrasaccharide unit (Fig. 6A), obtained as an acetolysis fragment, gives complete inhibition of the precipitin reaction while all other side chain fragments are much less effective (*34, 35*). The tetrasaccharide is unique in that it possesses a terminal α-$1 \rightarrow 3$-linked mannose unit; and the fact that the analogous α-$1 \rightarrow 3$-mannobiose is also a relatively good inhibitor (*34*) suggests that this is the immunodominant structure in bakers' yeast mannan. The backbone structure is not an important determinant in the mannan, since α-$1 \rightarrow 6$-linked mannooligosaccharides are poor inhibitors (*34, 36*). Furthermore, the removal of the side chains by digestion with the exo-α-mannanase leaves a backbone core that no longer precipitates with the homologous antiserum, nor does it inhibit the precipitation of native mannan (*35, 37*).

Employing a similar approach with other yeasts, we have found that the α-D-mannosyl phosphate group in *Kloeckera brevis* mannan (*26*) is the immunodominant structure of this yeast (Fig. 6B) (*38*). Mild acid hydrolysis of the phosphodiester mannan releases the labile mannose unit and the resulting phosphomonoester mannan reacts poorly with *Kloec. brevis* antiserum. Thus, the principal antigen is lost with removal of mannose from the phosphodiester structure. The data on inhibition support this contention, since α-D-mannose 1-phosphate is an excellent inhibitor compared with the other side chain fragments. *Saccharomyces cerevisiae* 4484-24D (*28*) and several brewing strains (*27*) possess a mannan chemotype that is indistinguishable from that of *Kloec. brevis*.

These results raise a question concerning the immunogenicity of the phosphate group in yeast mannans. In mannans of the *Kloec. brevis* chemotype, the phosphate is part of the major determinant (the mannosyl phosphate group), while the *S. cerevisiae* X2180 mannan contains phosphate in a similar but immunochemically unimportant structure (the mannobiosyl phosphate group). We conclude that the additional mannose unit in the phosphorylated side chain of X2180 mannan camouflages the phosphate group as far as its immunochemical reactivity is concerned.

Kluyveromyces lactis, and other yeasts of this genus, have another kind of antigenic side chain, the mannotetraose unit substituted by an *N*-acetyl-D-glucosamine residue (Fig. 6C) (*32*). However, this is not the sole determinant in this mannan, for the unsubstituted mannotetraose side chain (Fig. 6A) is also antigenic. The reaction of antiserums prepared against whole *Kluyv. lactis* cells with pure homologous mannan is inhibited to the same extent by the two side chains, but neither gives 100 percent inhibition. This suggests that there are two different antibody specificities in the serum, which is confirmed by the ability of both side chains together to give 100 percent inhibition. Moreover, as predicted, the adsorption of the antiserum to *Kluyv. lactis* cells with *S. cerevisiae* S288C mannan removes the antibody type that is specific for the mannotetraose side chain, but leaves the other activity unaffected. The homologous reaction with the adsorbed serum is completely inhibited by the pentasaccharide from the *Kluyv. lactis* mannan

(*35*). This same reaction can be analyzed in a different way by using as the antigen *Kluyv. lactis* mannan that has been digested with the exo-α-mannanase, during which all side chains are removed except for the one substituted by *N*-acetylglucosamine. The precipitin reaction with this mannan is completely inhibited by the pentasaccharide side chain, and is unaffected by the mannotetraose unit (*32*).

Like *Kluyv. lactis* mannan, *S. italicus* has a pentasaccharide side chain. However, this structure (Fig. 6D) does not appear to be antigenically different from the mannotetraose side chain, and our studies indicate that the serotype is indistinguishable from that of *S. cerevisiae* S288C (*30*).

The variety of patterns obtained in the original acetolysis analyses (*22*), suggested that there was a high order of species specificity in the mannan structures. However, the patterns alone do not tell the whole story, because it is obvious that the fine structure of the fragments must be important, particularly the anomeric configurations and the linkages between the mannose units as well as the presence of other substituents such as phosphate and hexosamine. Moreover, some mannans appear to have what has been called a "block type" structure of more or less linear molecules formed by blocks of 1→2- and 1→3-linked mannose units connected by 1→6 linkages (*24*). Acetolysis of such a mannan would appear to yield an assortment of "side chains," which in fact would be derived from the backbone of the polymer. However, even these mannans can be characterized through the combined analysis by exo-α-mannanase treatment, acetolysis, and methylation (*25*).

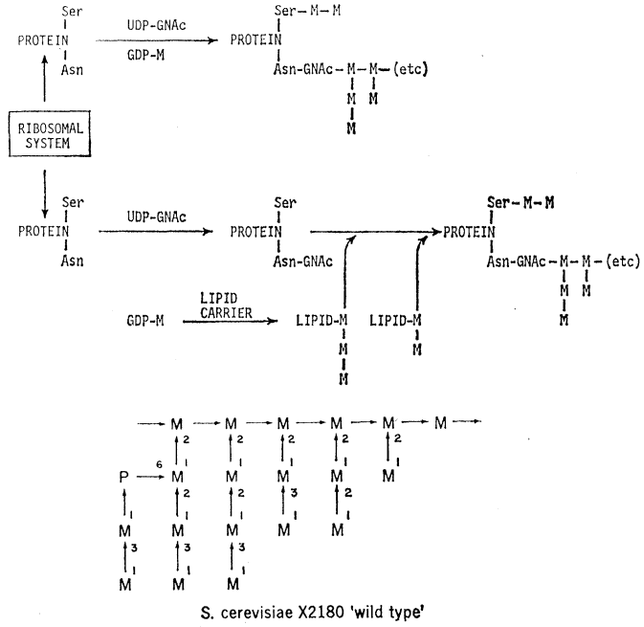
Among the most complicated acetolysis patterns are those given by mannans from *Candida* species, in which fragments of at least seven mannose units in length are obtained. It has been shown that these long side chains are the immunodominant structures in *C. albicans* mannans, and it has been possible to distinguish fine differences between serotypes A and B which are correlated with the lengths of the side chains and the relative amounts of 1→2 and 1→3 linkages (*39*). All of these points suggest that the difference between these two serotypes lies in some aspect of mannan structure.

Genetic Control of Mannan Structure

Little is known about the genetic control of mannan structure or the mechanism of its biosynthesis. Since the mannan is a polysaccharide-protein complex, it is not surprising that its formation can be inhibited by cycloheximide (*40*). On the other hand, whether the mannose units are added one at a time to the growing chain as in glycogen synthesis, or are built into fragments on a carrier and then polymerized as in the synthesis of the bacterial O-antigen lipopolysaccharide, has not been determined. Evidence for a mannosyl-lipid intermediate in mannan synthesis has been obtained, but lipid-bound oligosaccharides have not been detected (*41*). At present, both of the pathways outlined in Fig. 7 appear to be equally likely.

A comparison of the four structures in Fig. 5 suggests that there is a certain unity in the biosynthesis of these yeast mannans. In addition to a common α-1→6-linked polymannose backbone, the first side chain mannose unit in each is attached exclusively by an α-1→2 linkage. Thus, it seems probable that the biosynthetic steps are similar in all of these yeasts, and that the distinctive differences can be explained by a few steps representing one or two enzymatic differences.

We have recently been able to learn something about the genetic control of mannan structure by studying crosses between haploid strains with different mannan chemotypes. As pointed out in Fig. 5, *S. cerevisiae* X2180 possesses mannan with the immunodominant α-1→3-linked terminal mannose unit. On the other hand, *S. cerevisiae* 4484-24D possesses the α-D-mannosyl phosphate chemotype characteristic of *Kloec. brevis* mannan. Mannans of these two chemotypes can be distinguished easily by their serological reactions, and the agglutination by specific antiserums can be used to assay for



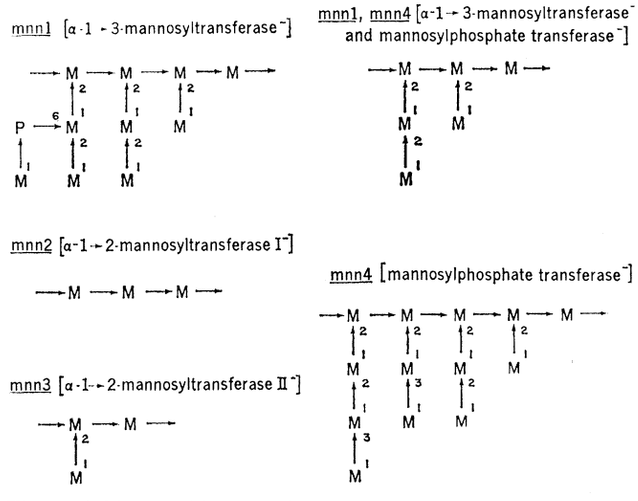


Fig. 7 (left). The biosynthesis of mannan may occur by formation of the protein acceptor, to which the sugars are attached in a stepwise manner. Alternatively, oligosaccharides may be built up on a lipid carrier before being transferred to the growing chain with the formation of the 1→6-linked backbone. (UDP, uridine diphosphate; GDP, guanosine diphosphate; other abbreviations as in Fig. 2.) Fig. 8 (right). Structures of the mannans formed by the various yeast mannan mutants which appear to be defective in one of several mannosyltransferases or in a mannosylphosphate transferase. When crossed to form hybrid diploids, all of the mutants complement each other so that the wild-type X2180 mannan shown at the top is formed.

the kind of mannan that is present on the surface of hybrids. The general procedure is to prepare complementing auxotrophs of haploid strains of opposite mating type so that hybrids can be isolated from a mixed culture by their ability to grow on minimal medium. The hybrid from a cross between *S. cerevisiae* X2180-1A (*a* mating type) and 4484-24D (α mating type) agglutinated with antiserum to *S. cerevisiae* X2180 cells but not with antiserum to 4484-24D cells, and apparently produced X2180-type mannan rather than a mixture of the two different chemotypes. Furthermore, the mannan isolated from the hybrid exhibited properties of the X2180 strain.

The hybrid was subjected to tetrad analysis so that the gene that controls the mannan chemotype could be mapped (*28*). The analysis revealed that the two mannan chemotypes segregate in a ratio of $2^+ : 2^-$, and that the gene determining the X2180-type mannan is centromere linked. The controlling gene, designated *mnn*1, is located on chromosome V near to the uracil-3 (*ura*3) locus. This is the first gene concerned with mannan biosynthesis to be placed on the genetic map of yeasts. From the differences between the mannans produced by strains X2180-1A and 4484-24D, it was concluded that this gene must be involved in formation of an α-1 → 3-mannosyltransferase. In addition to having a role in the formation of the mannotetraose side chain, this enzyme also modifies expression of the presumed mannosylphosphate transferase that is contributed to the hybrid by the 4484-24D genome. This appears to result from the ability of the α-1 → 3-mannosyltransferase to convert mannosylphosphate groups to α-1 → 3-mannobiosylphosphate groups (*30*). To extend this study of the genetic control of mannan structure, we have investigated mannan mutants of the two *S. cerevisiae* strains.

Mannan mutants of S. cerevisiae. In our studies of mannan mutants we have two aims: (i) to delineate the pathway and control of mannan biosynthesis, and (ii) to determine the role of species specific differences in mannan structure. We have employed specific antiserums for the selection of mannan mutants from *S. cerevisiae* cultures treated with ethyl methane sulfonate (*42*). Antiserum specific for the α-1 → 3 linkage of the tetrasaccharide side chain was prepared by adsorbing cross-reacting antibodies from antiserum to X2180 cells with *Kloec. brevis* cells. Selection of mannan mutants from a mutagenized culture involved the removal of wild-type cells by agglutination with the specific antiserum. Nonaggregated cells were cultured on agar plates and individual colonies were screened for decreased agglutinability.

Three kinds of X2180 mannan mutants were obtained and are shown in Fig. 8. One lacks only the terminal α-1 → 3-linked side chains, from which we conclude that the genetic alteration must be in the α-1 → 3-mannosyltransferase gene already designated the *mnn*1 locus from an earlier study (*28*). Another group of mutants is defective in the formation of either of two α-1 → 2-mannosyltransferases (*mnn*2 and *mnn*3). One class (*mnn*2 mutants) produces mannan with an unsubstituted α-1 → 6-linked backbone, and presumably it lacks the α-1 → 2-mannosyltransferase that is needed to attach the first side chain mannose unit. The other (*mnn*3) yields predominantly mannose and mannobiose upon acetolysis of the mannan, and presumably it has lost a second α-1 → 2-mannosyltransferase that is required for attachment of the second side chain mannose unit. Structural analyses of the isolated polysaccharides confirmed the kinds of modifications described.

The *mnn*1 mutant has been particularly useful for investigating the dominance of the X2180 chemotype in the hybrid with 4484-24D. We find that the presence of an active α-1 → 3-mannosyltransferase is alone responsible for the suppression of the 4484-24D chemotype, since a hybrid of X2180 *mnn*1 and 4484-24D possesses the mannosylphosphate determinant that is characteristic of the 4484-24D chemotype. Furthermore, the *mnn*1 mutant itself agglutinates well with antiserum to 4484-24D cells, which indicates that the X2180 wild-type strain must carry the mannosylphosphate transferase in a cryptic or an unexpressed form. In fact, as already mentioned, the X2180 wild-type mannan contains α-1 → 3-mannobiosylphosphate units rather than the mannosylphosphate groups of 4484-24D mannan. Thus, the *mnn*1 locus, which is responsible for the dominance of the X2180 chemotype, codes for an α-1 → 3-mannosyltransferase that apparently utilizes both mannotriose side chains and mannosylphosphate groups as acceptors.

Using *Kloec. brevis* antiserum, which is specific for the mannosylphosphate determinant, we have selected a mutant of 4484-24D that lacks this structure (*43*). The mannan isolated from this mutant (Fig. 8) contains very little phosphate, and acetolysis yields mannose, mannobiose, and mannotriose, but no phosphorylated fragment. This mutation is assumed to be in a fourth mannan locus, designated *mnn*4, that codes for a mannosylphosphate transferase.

Each of the four types of mutants is able to complement with the other mutant strains to yield hybrid diploids that produce wild-type X2180 mannan (*43*). Our studies indicate that the wild-type 4484-24D strain is very similar to the *mnn*1 mutant of X2180-1A. Therefore, the mutant of 4484-24D that lacks the mannosylphosphate transferase activity is considered to be doubly defective, containing alterations in both the *mnn*1 and *mnn*4 loci. In the cross of X2180-1A *mnn*2 and 4484-24D *mnn*1, the wild-type X2180 mannan is produced, so that the 4484-24D genes (in this case, the *mnn*2 locus) must be expressed in a hybrid formed with an X2180 strain.

Other studies on the genetics of the yeast cell surface. Some *S. cerevisiae* strains have the property of binding alcian blue, a dye of the phthalocyanine type. The binding ability is associated with the mannan component of cell wall extracts and seems to be correlated with the fractions of higher phosphate content. Genetic analysis has shown that the dye binding ability is controlled by a single gene that is linked to the *ura*1 locus (*44*), a gene that has not yet been placed on a specific chromosome. We considered it possible that dye binding might also be a property of *S. cerevisiae* 4484-24D, which has a phosphomannan in its cell wall, and that strains lacking the mannosylphosphate group might fail to bind the dye. Indeed, the strains that express the mannosylphosphate determinant (X2180 *mnn*1 mutants and 4484-24D) do bind the dye strongly, while the other strains bind the dye weakly. In fact, the X2180 *mnn*2 mutants, which lack all side chains, and the 4484-24D *mnn*4 mutants, which synthesize no phosphorylated side chains, do not bind the dye at all. The hybrid diploid from a cross of these two nonbinding mutants exhibits the dye binding property of the wild type X2180.

The studies of Friis and Ottolenghi

(*44*) suggest that the dye binding property is not dependent solely on the presence or absence of phosphate, but that the binding may be sensitive both to the location of the phosphate and the nature of its linkage in the mannan. While we now can say for certain that phosphate is required for dye binding, some of our results do support the ideas of Friis and Ottolenghi. For example, we have already indicated that the wild-type strain X2180 does not possess mannosylphosphate groups on the cell surface or in the mannan which can be detected by the immunochemical methods we have employed. Yet, the mannan does contain approximately half as much phosphate as strain 4484-24D, in the form of mannobiosylphosphate groups, and it does bind alcian blue to a variable extent. We are forced to the conclusion that the concentration, location, and type of mannosylphosphate groups in the mannan have an important effect on the dye binding ability as well as on the agglutinability of the cells. These observations are reminiscent of those made on the changed agglutinability of normal animal cells following viral transformation (*45*). In that case, the changes may be correlated with a reorganization of the binding sites on the cell surface as well as with a change in the total number of sites.

In another study closely related to our own, Spencer *et al.* (*46*) have analyzed a hybrid between two *S. cerevisiae* strains whose mannans were distinguished on the basis of their NMR spectra. One mannan was of the X2180 type, while the other lacked the $1 \rightarrow 3$ linkage and had a mannan of the 4484-24D type (*28, 30, 47*). It was observed that the hybrid diploid formed a mannan that possessed the $1 \rightarrow 3$ linkage, and that the gene controlling this characteristic feature was not linked to *ade*1, *his*2, *his*7, *leu*1, *ura*1 (*ade*, adenine; *his*, histidine; *leu*, leucine; *ura*, uracil). This is probably the same gene we have mapped (*mnn*1,) and in agreement with our results none of the above markers is on chromosome V.

The Physiological Role of Yeast Mannan-Proteins

There appear to be two classes of mannan-proteins, one including those with a structural role that are composed 90 percent or more of mannose and 5 to 10 percent of protein or peptide material; and another including the mannan-protein enzymes, which are composed of 50 to 70 percent mannan. The mannan-enzymes, among which are invertase, melibiase, glucosidase, and phosphatase, may be embedded in a matrix formed by the structural mannan-protein. In the case of *Kloec. brevis*, the structural mannan-protein appears to be constructed from subunits, each subunit having a molecular weight of about 25,000 and being composed of about 150 mannose and 20 amino acid units which are cross-linked into larger structures (Fig. 2) (*48*). A similar arrangement of mannan-protein is observed in *Kluyv. lactis* (*32*).

A species specific surface polysaccharide of the mannan-protein type might have one of several different roles. It could participate in recognition for mating, or in a specific agglutination reaction as in *Hansenula wingei* (*5*), or it could serve as an organizational template for the ordered arrangement of the mannan-protein enzymes in the cell wall. If any of these functions were dependent on the fine structure of mannan, one would expect mutants, particularly of the *mnn*2 type which contain only the backbone structure, to be seriously affected. On the contrary, this mutant is able to grow and mate with normal efficiency. However, it may be significant that no mutant was obtained that lacked mannan. From the biosynthetic pathways shown in Fig. 7, it would appear that an alteration in any of a number of enzymes should result in the loss of mannan production. Attempts to obtain mutants of X2180 *mnn*2 that fail to make the α-$1 \rightarrow 6$-linked backbone structure have yielded only revertants to the wild type X2180 (*30*). Genetic studies on the sexual agglutination factor of *H. wingei* have shown that the type specificity is closely linked to the mating type locus, and other studies indicate that the agglutination factors are mannan-proteins in which the specificity of reaction appears to reside in the protein parts (*5*). Still it is possible that the activity of the agglutination factors might be affected by mutations in the mannan structure. In general, however, we find that modification in the mannan structure has surprisingly little effect on the physiological properties of the yeast cell, a result that makes it even more puzzling why yeast mannans occur in such variety and show the species specificity we have described.

Summary

Most of the common yeasts have a cell wall composed of a rigid glucan layer and a mannan-protein complex which coats and is interspersed with the glucan. The mannan-protein is a mixture of macromolecules in which the polysaccharide component makes up 50 to 90 percent of the weight of the particular fraction, and is attached covalently to the protein part. Different mannan-protein fractions have different functions, some being structural, some being mannan-enzymes, and some serving for recognition and specific agglutination. Other undetermined functions no doubt exist.

The mannan-protein is the principal immunogen of the yeast cell, probably because it is exposed on the cell surface. The carbohydrate side chains of the mannan seem to possess most of the antigenic activity, since fragments obtained by acetolysis of the polysaccharide often give complete inhibition of the precipitin or agglutination reactions with homologous antiserum.

While immunochemical cross-reactivity between yeasts is not unusual, most yeasts do possess species specific antigens. This species specificity is also revealed in the patterns produced by acetolysis of mannans; such patterns reflect gross differences between the structures of different yeast mannans. From a detailed analysis of the acetolysis fragments and from other studies, it has been possible to define the structures of the polysaccharide component of several yeasts. All show fine structural features consistent with the observed immunochemical similarities and differences.

Hybrid diploids obtained by crossing haploid *Saccharomyces* strains that have different mannan chemotypes are fully complementary in the sense that the kind of mannan made by the diploid is controlled by interaction of the genes carried by the two different haploids. The enzymic basis for this regulation of mannan structure was elucidated by a study of yeast mannan mutants. Four classes of *S. cerevisiae* mutants have been obtained which make altered mannans, and the mutant genes are implicated in the formation of three mannosyltransferases and a mannosylphosphate transferase, all of which appear to function in synthesis of the side chains of the mannan molecule. These mutants should be useful in working out the mechanism of man-

nan biosynthesis and in defining the physiological role of the mannan component of the mannan-protein complexes.

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29. Because *S. cerevisiae* 4484-24D was of central importance for our genetic studies, we attempted to determine its history. Our culture was obtained from S. Fogel (University of California, Berkeley) who had isolated it as a nontemperature-sensitive spore from a cross between X2180-1B(α) and strain TS-171(*a*), the latter coming from L. Hartwell (University of Washington, Seattle). Hartwell says that TS-171 was derived from strain A364A that he obtained from S. Esposito in Herschel Roman's laboratory (University of Washington, Seattle). Roman reported that his cultures trace back mainly to strains developed by C. C. Lindegren (Southern Illinois University, Carbondale). Since "wild" strains have been found which possess the same phosphomannan chemotype, for example those from Guinness Laboratories (*27*), we think that the 4484-24D mannan type has not resulted from an unrecognized mutation of a culture with the S288C-type mannan during the laboratory manipulations to which the strain has been subjected over the years. Strain A364A has the same phosphomannan chemotype as 4484-24D.
30. Unpublished data.
31. According to R. K. Mortimer (University of California, Berkeley), strain S288C was first isolated as an α-haploid. Later it changed spontaneously to an *a*/α-diploid, which consequently was isogenic with the exception of the mating type locus. This diploid was sporulated to yield the haploids of opposite mating type, now designated X2180-1A(*a*) and X2180-1B(α).
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49. We thank the students and colleagues who, since the beginning of this project, have contributed many of the facts and ideas that provide the basis of this article. We are indebted to Professors S. Fogel and R. K. Mortimer for assistance in the genetic analysis of yeast mannan mutants. The work was supported by NSF grants GB-19199 and GB-35229X, and by PHS grant AM884.

Food-Related Energy Requirements

The energy used by the U.S. food cycle constitutes about 12 percent of the national energy budget.

Eric Hirst

In this article I discuss the quantities of energy required to grow, process, transport, wholesale, retail, refrigerate, and cook food in the United States for the year 1963, and use the data available for that year (*1*) to estimate the annual energy consumption for food during the period 1960 to 1970. Energy requirements per unit of food energy and of food protein are computed for the major food groups.

Because food accounts for 20 percent of disposable personal income in the United States, it seems likely that the energy used in moving food through the economy also comprises a sizable percentage of the total U.S. energy budget. The study I describe here was initiated as a result of the energy problems now facing the nation: fuel shortages, rapidly rising fuel prices, brownouts, adverse environmental impacts of fuel cycles, and continued growth in energy demand. In this study, an analysis was made of the impact of food on energy consumption, this being a necessary first step in evaluating methods to increase the efficiency of energy use in delivering food to consumers. The results obtained can also be used to assess the impact of increased fuel prices on food prices.

Personal consumption expenditures (PCE) (*2*) for food totaled $132 billion in 1970 (*3*). Between 1960 and 1970 the percentage of food dollars spent away from home (eating out) increased from 20 percent to 22 percent. This, plus a shift to more expensive food, accounted for an increase in food expenses. Although expenses rose dur-